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STUDIES ON THE METABOLISM OF CEREAL GRAINS

II. THE EFFECT OF AGE AND KERNEL SIZE ON THE COURSE OF RESPIRATION OF WHEAT DURING EARLY GERMINATION STAGES[1]

BY WILLIAM LEACH[2]

Abstract

Wheat samples that had been previously stored for periods of 6, 18, and 30 months were kept at 25° C. (*a*) in contact with water, and (*b*) in a moisture-saturated atmosphere for 40-hr. periods during which hourly records of their carbon dioxide outputs were made. The lengths of the previous storage periods did not appear to have had any significant effect on the respiratory activities of the samples. Kernel size, however, was found to have a definite effect on respiration on the basis of the quantity of carbon dioxide produced per unit weight of grain, kernels of large average size giving a lower carbon dioxide output per unit weight of grain than kernels of small average size.

In the course of the investigations described in the preceding paper of this series (2) the question arose as to whether any marked variation in the respiratory behaviour of wheat samples might occur as a result of variations in the lengths of time that had elapsed between the harvesting of the grain and its germination. In consequence of this possibility a number of similar experiments were carried out using series of wheat samples that had respectively been kept in storage for periods of approximately 6, 18, and 30 months.

In addition to normal germination experiments in which the course of respiration was determined with the grain in contact with water, a number of experiments were carried out in which the grain absorbed water entirely from the surrounding air which was maintained at 100% relative humidity. In this way respiration data were obtained with the wheat under conditions somewhat more comparable to, though obviously not identical with those obtaining when the grain is in storage.

Materials and Methods

For these experiments Dr. Craigie of the Dominion Rust Laboratory, Winnipeg, provided three series of samples of wheat of varieties Renown, Marquis, and Thatcher, respectively. For each of the above varieties there were samples harvested in the summers of 1939, 1940, and 1941, all of which had been kept under identical storage conditions.

[1] *Manuscript received June 11, 1943.*

Contribution from the Department of Botany, University of Manitoba, Winnipeg, Man., with financial assistance from the National Research Council of Canada and the University of Manitoba Research Committee.

[2] *Professor of Botany, University of Manitoba.*

Respiration determinations were made and automatically recorded with the katharometer apparatus (3). In the normal germination experiments single wheat kernels were used and were placed in the respiratory plant chamber in contact with water as previously described (2). In the experiments in which absorption of water by the grain was entirely from the air contained in the plant chamber, which was kept in a moisture-saturated state, six grains were used for each run, and the arrangement used was that shown in Fig. 1.

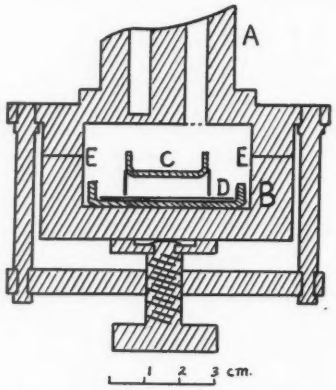

FIG. 1. *Vertical section through katharometer plant chamber. For description see text.*

In this figure the katharometer block and the solid brass plant chamber are respectively shown in section at *A* and *B*. These two are connected together with a gas-tight ground joint indicated at *E*, *E*, anhydrous lanolin being used as a sealing material and close contact maintained between the ground surfaces with the screw-clamping device shown. Within the plant chamber the experimental grain was placed in a small glass dish, *C*, which was supported on a wire tripod standing in another dish, *D*. The humidity of the air in the plant chamber was raised to saturation point and so maintained by distilled water added to a disk of filter paper lying on the bottom of the lower dish, *D*, before sealing up.

As in previous experiments the whole apparatus was immersed in a large thermostatically controlled water-bath maintained at a temperature of 25° C.

Respiratory Changes During Normal Germination with the Grain in Contact with the Water

The course of respiration during the early germination period extending over approximately 40 hr. is shown for each sample of the three varieties of wheat used in Tables I, II, and III and in Figs. 2, 3, and 4. In these tables the hourly amounts (in milligrams) of carbon dioxide evolved by the germinating seeds are recorded at three-hour intervals throughout each experimental

run. Two sets of figures, with corresponding graphs, are given for each experiment, namely, milligrams of carbon dioxide per gram fresh weight of grain, and milligrams of carbon dioxide per kernel. From an examination of the former one might be led to conclude that the age of the grain sample has a marked influence on its respiration intensity. In all three varieties, the

TABLE I

RESPIRATORY BEHAVIOUR OF "RENOWN" WHEAT DURING GERMINATION IN CONTACT WITH WATER AT 25° C. AFTER STORAGE FOR 6, 18, AND 30 MONTHS

1939 Crop Wt. of grain = 0.0262 gm.			1940 Crop Wt. of grain = 0.0236 gm.			1941 Crop Wt. of grain = 0.042 gm.		
Germination time, hr.	Respiration rate, mg. CO_2 per hr.		Germination time, hr.	Respiration rate, mg. CO_2 per hr.		Germination time, hr.	Respiration rate, mg. CO_2 per hr.	
	Per gram	Per kernel		Per gram	Per kernel		Per gram	Per kernel
8.5	0.63	0.017	8.5	0.62	0.015	8.5	0.35	0.015
11.5	0.70	0.018	11.5	0.75	0.018	11.5	0.42	0.018
14.5	0.87	0.023	14.5	0.95	0.022	14.5	0.60	0.025
17.5	0.96	0.025	17.5	1.17	0.027	17.5	0.87	0.034
20.5	1.10	0.029	20.5	1.27	0.030	20.5	0.94	0.040
23.5	1.23	0.032	23.5	1.57	0.037	23.5	0.94	0.040
26.5	1.39	0.037	26.5	1.92	0.045	26.5	1.14	0.047
29.5	1.84	0.048	29.5	2.17	0.051	29.5	1.36	0.057
32.5	2.02	0.053	32.5	2.47	0.058	32.5	1.43	0.060
35.5	2.18	0.057	35.5	2.69	0.064	35.5	1.72	0.072
38.5	2.47	0.065	38.5	2.77	0.065	38.5	1.90	0.080
41.5	2.65	0.070	41.5	3.07	0.073	41.5	2.11	0.089

TABLE II

RESPIRATORY BEHAVIOUR OF "MARQUIS" WHEAT DURING GERMINATION IN CONTACT WITH WATER AT 25° C. AFTER STORAGE FOR 6, 18, AND 30 MONTHS

1939 Crop Wt. of grain = 0.0270 gm.			1940 Crop Wt. of grain = 0.0354 gm.			1941 Crop Wt. of grain = 0.0380 gm.		
Germination time, hr.	Respiration rate, mg. CO_2 per hr.		Germination time, hr.	Respiration rate, mg. CO_2 per hr.		Germination time, hr.	Respiration rate, mg. CO_2 per hr.	
	Per gram	Per kernel		Per gram	Per kernel		Per gram	Per kernel
13.5	0.21	0.019	13.5	0.46	0.016	12.5	0.51	0.019
16.5	0.25	0.022	16.5	0.80	0.028	15.5	0.54	0.021
19.5	0.25	0.022	19.5	0.94	0.032	18.5	0.62	0.024
22.5	0.28	0.025	22.5	0.97	0.034	21.5	0.79	0.030
25.5	0.32	0.028	25.5	1.06	0.037	24.5	0.88	0.034
28.5	0.39	0.035	28.5	1.28	0.044	27.5	1.08	0.041
31.5	0.41	0.037	31.5	1.48	0.051	30.5	1.27	0.048
34.5	0.53	0.047	34.5	1.67	0.059	33.5	1.44	0.055
37.5	0.54	0.048	37.5	1.84	0.064	36.5	1.75	0.067
40.5	0.61	0.055	—	—	—	39.5	1.82	0.069

TABLE III

RESPIRATORY BEHAVIOUR OF "THATCHER" WHEAT DURING GERMINATION IN CONTACT WITH WATER AT 25° C. AFTER STORAGE FOR 6, 18, AND 30 MONTHS

1939 Crop Wt. of grain = 0.0326 gm.			1940 Crop Wt. of grain = 0.0324 gm.			1941 Crop Wt. of grain = 0.0355 gm.		
Germination time, hr.	Respiration rate, mg. CO_2 per hr.		Germination time, hr.	Respiration rate, mg. CO_2 per hr.		Germination time, hr.	Respiration rate, mg. CO_2 per hr.	
	Per gram	Per kernel		Per gram	Per kernel		Per gram	Per kernel
12.0	0.45	0.015	13.5	0.54	0.018	12.5	0.19	0.013
15.5	0.60	0.019	16.5	0.69	0.022	15.5	0.22	0.015
18.5	0.63	0.021	19.5	0.82	0.029	18.5	0.26	0.018
21.5	0.81	0.026	22.5	0.94	0.031	21.5	0.31	0.021
24.5	0.99	0.032	25.5	1.15	0.037	24.5	0.37	0.025
27.5	1.09	0.036	28.5	1.39	0.045	27.5	0.39	0.026
30.5	1.35	0.044	31.5	1.65	0.053	30.5	0.50	0.034
33.5	1.72	0.056	34.5	1.75	0.057	33.5	0.58	0.039
36.5	1.97	0.064	37.5	2.12	0.066	36.5	0.71	0.048
39.5	2.08	0.068	—	—	—	39.5	0.99	0.068

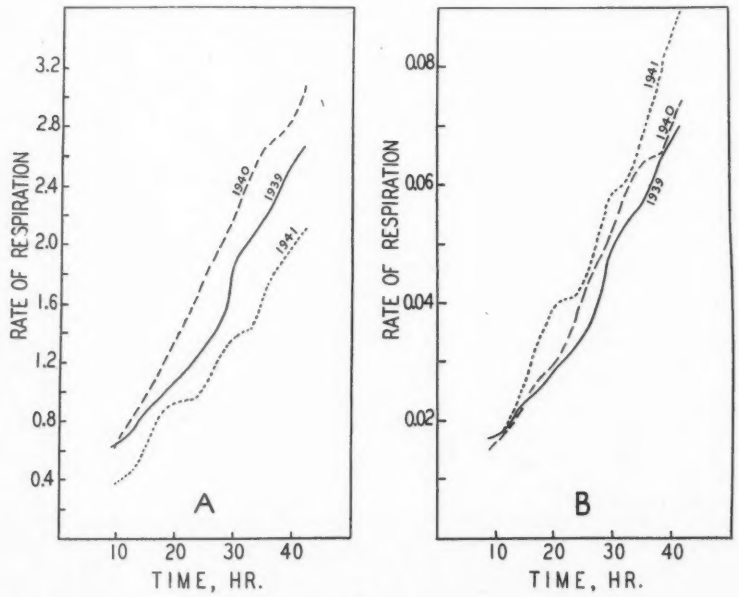

FIG. 2. *Respiration of "Renown" wheat of three crop years during early germination, in contact with water, at 25° C.*

A. Respiration as milligrams carbon dioxide per gram initial weight of grain per hour.

B. Respiration as milligrams carbon dioxide per kernel per hour.

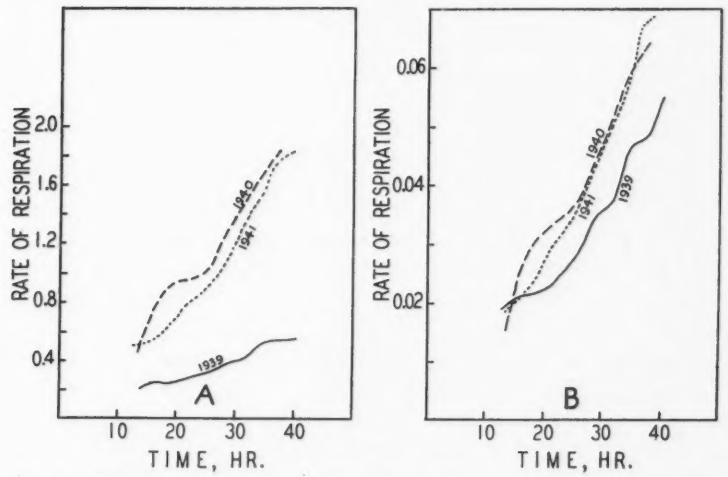

FIG. 3. *Respiration of "Marquis" wheat of three crop years during early germination, in contact with water at 25° C.*
Respiration rates as denoted in Fig. 2, A and B.

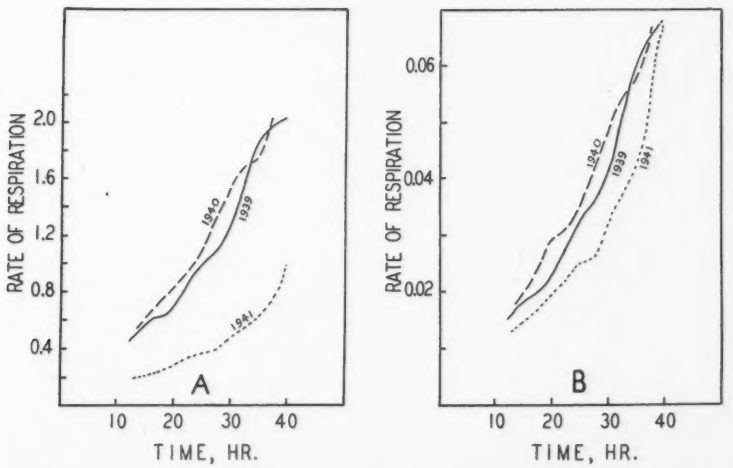

FIG. 4. *Respiration of "Thatcher" wheat of three crop years during early germination, in contact with water, at 25° C.*
Respiration rates as denoted in Fig. 2, A and B.

1940 sample appears to have the highest respiration rate while the lowest rate is shown by the 1941 samples of Thatcher and Renown and by the 1939 sample of Marquis. Such a variation in behaviour between varieties though conceivably possible seems highly improbable, and the solution to the mystery

becomes evident if the numbers and graphs for carbon dioxide output per kernel are examined. It will be observed that when this unit is used to denote the respiration rate of the grain, the remarkable varietal differences referred to above either disappear or become much less definite. As far as respiratory behaviour during germination goes, one may therefore conclude that during the first three years of storage at least, the length of the storage period has no significant effect on the respiration of the grain.

A further point of interest that comes out of these experiments is that they furnish definite confirmation of the view expressed by Bailey and Gurjar (1) that the seat of respiratory activity of the wheat grain lies mainly in the embryo and that the endosperm plays a relatively small part in the direct production of carbon dioxide.

The Respiratory Changes Shown by Grain While Absorbing Water from a Saturated Atmosphere

In the following experiments the course of respiration was followed during the period when the grain was taking up water from moisture-saturated air. The results of these experiments may in some ways be considered as furnishing a slow motion picture of the initial stage of germination which under normal conditions with the seed in contact with water is passed through at an extremely rapid rate.

For this investigation the one variety "Renown" was used, experimental runs of 40 hr. being carried out for each of the three samples. The numerical data for the experiments are given in Table IV. Here also the respiration rates are given as calculated on a basis of carbon dioxide per gram per hour and on a basis of carbon dioxide per kernel per hour.

TABLE IV

RESPIRATORY BEHAVIOUR OF "RENOWN" WHEAT WHILE ABSORBING WATER FROM MOISTURE-SATURATED AIR AT 25° C. AFTER STORAGE FOR 6, 18, AND 30 MONTHS

Germination time, hr.	Mg. CO_2 per gram per hour			Mg. CO_2 per kernel per hour		
	Crop 1939 Expt. 130	Crop 1940 Expt. 132	Crop 1941 Expt. 131	Crop 1939 Expt. 130	Crop 1940 Expt. 132	Crop 1941 Expt. 131
10	0.068	0.058	0.042	0.0021	0.0021	0.0016
13	0.074	0.060	0.047	0.0023	0.0022	0.0018
16	0.083	0.076	0.062	0.0025	0.0027	0.0024
19	0.089	0.076	0.065	0.0027	0.0027	0.0025
22	0.089	0.063	0.060	0.0027	0.0023	0.0023
25	0.098	0.071	0.067	0.0030	0.0026	0.0025
28	0.117	0.092	0.087	0.0036	0.0033	0.0033
31	0.153	0.113	0.107	0.0047	0.0041	0.0041
34	0.190	0.144	0.142	0.0058	0.0052	0.0054
37	0.215	0.168	0.174	0.0066	0.0060	0.0066
40	0.233	0.207	0.214	0.0071	0.0075	0.0081

NOTE:—*The weights of the six kernels used in each of the above experiments were:*
Expt. 130 (1939), 0.183 gm.
Expt. 132 (1940), 0.214 gm.
Expt. 131 (1941), 0.226 gm.

Again no significant differences in the respiration rates could be detected between the samples in consequence of their having been stored for periods of 6, 18, and 30 months, respectively.

Further, the same evidence of the main respiratory activity being seated in the embryo is observable when a graphic representation of the experimental data expressed as carbon dioxide per gram is compared with one expressed as carbon dioxide per kernel. This is evident if Fig. 5A is compared with Fig. 5B.

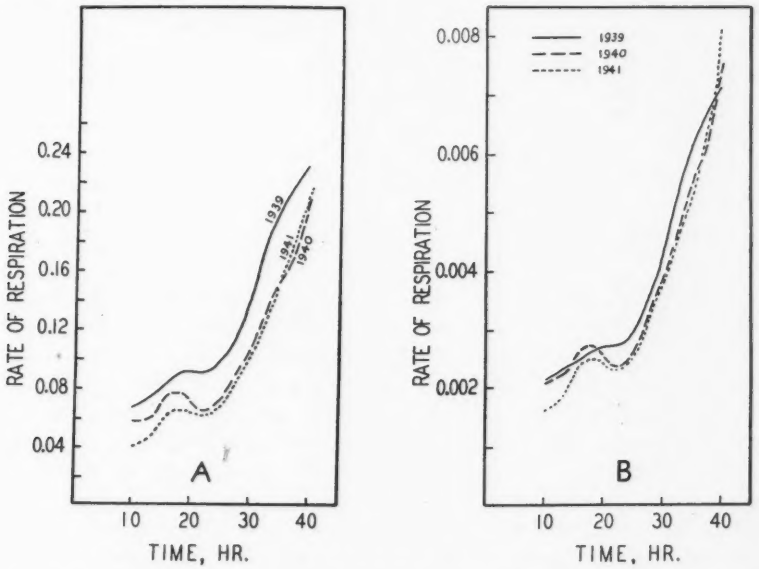

FIG. 5. *Respiration of "Renown" wheat during early germination, while absorbing water from moisture-saturated air, at 25° C.*
Respiration rates as denoted in Fig. 2, A and B.

With regard to the developmental stage reached by the grains at the end of the experimental period in this investigation as compared with the normal germination experiments described in the preceding section of this paper. It will be noted that "Renown" wheat absorbing water from saturated air reaches a respiratory activity at the end of 40 hr. that is only about half that reached after eight hours when germination in contact with water occurs in the normal way (see Figs. 2 and 6). The difference in behaviour is obviously largely due to the limiting effect of the water content of the grain in the moist air experiments. In both sets of experiments the initial water content of the grain was 10%. In the experiments with the grain in contact with water, the first 10 hr. resulted in the water content of the grain rising to a value of approximately 35%, whereas, in the moist air experiments, in

40 hr. a water content of approximately only 28% was reached. This last fact is demonstrated by the graph shown in Fig. 6 which gives the rate at which water is absorbed by "Renown" wheat from moisture-saturated air

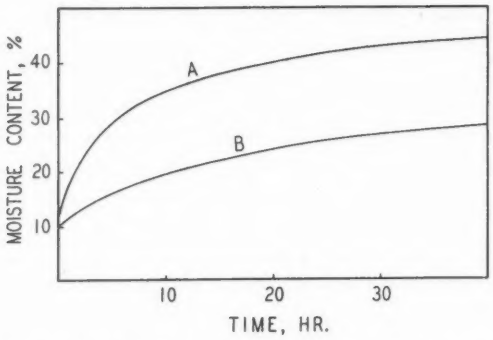

FIG. 6. *Water absorption graphs for "Renown" wheat at 25° C. A, grain in contact with water; B, grain in moisture-saturated air.*

when under conditions identical with those used when the carbon dioxide output measurements were being made and also the water absorption of the same variety when in contact with water.

Conclusions

From these experiments it would thus appear probable that, provided other conditions remain constant, the length of the storage period would not have any marked influence on the tendency of grain to heat, that is, so far as the heating may be due to the respiration of the grain itself. On the other hand, the average size of the individual kernels of any particular mass of grain will have a very definite influence on the total amount of carbon dioxide produced by that mass in unit time. A small average kernel size might therefore be accompanied by a greater tendency to heat than would a large average kernel size. It may be pointed out here that the grain used in the above described experiments had been stored for the periods mentioned under ideal conditions.

Acknowledgment

I am indebted to Mr. D. R. Moir for the provision of the data relating to the rate of water absorption of Renown wheat from a moisture-saturated atmosphere.

References

1. BAILEY, C. H. and GURJAR, A. M. J. Agr. Research, 12 : 685-713. 1918.
2. LEACH, W. Can. J. Research, C, 20 : 160-168. 1942.
3. STILES, W. and LEACH, W. Ann. Botany, 45 : 461-488. 1931.

THE EFFECT OF TEMPERATURE DIFFERENTIAL ON THE MOISTURE CONTENT OF STORED WHEAT[1]

By J. Ansel Anderson[2], J. D. Babbitt[3], and W. O. S. Meredith[4]

Abstract

Local increases in the moisture content of dry wheat stored in country elevator annexes have occasionally been observed in Western Canada. A laboratory experiment suggests that the chief cause is a temperature differential established during the winter. The air in the warmer parts of the grain contains a greater quantity of water vapour than that in the colder, and moisture is transferred either by diffusion or by the convective movement of the air as a whole. A temperature difference of 35° C., across 6 ft. of grain having an initial moisture content of 14.6%, caused the moisture content at the cold end (0° C.) to rise to over 20% in 316 days. The experiment indicates that this movement of moisture is a slow process and that equilibrium conditions are never established for any practical length of time or mass of wheat.

In 1940 and 1941 it became necessary to build a large number of annexes to country elevators in order to accommodate the enormous amounts of grain accumulating in Canada. The average annex was a rectangular wooden frame building with shed roof, 60 ft. long by 30 ft. wide, 20 ft. high at the eaves, and 35 ft. high at the ridge. Sides and ends were made of ship-lap with a layer of oiled paper on the inside; various types of roofing material were used. Most of the annexes had no bulkhead, and held about 30,000 bushels in one pile. They were generally filled in the fall with sound high grade wheat of low moisture content. At first many of them were filled to within four feet of the ridge. The buildings were designed to keep out driving rain and snow; in consequence no provision was made for ventilation above the wheat.

In the spring, trouble occurred in a number of annexes because a layer of damp grain 1 to 2 ft. deep had developed at or near the surface of the grain. Moisture contents of 16 to 18% in this layer were not uncommon, and the damp grain was generally heavily infested with mites. The trouble was cured by removal of the damaged grain and by fumigation of the remainder when this additional step seemed advisable. Prevention methods, developed by guesswork, involved lowering the load line, and providing some means of ventilation that could be closed during rain and snow storms. Careful watch was kept on the grain, and, if the moisture content started to increase near the surface, the grain was shovelled over and thus dried.

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[3] *Physicist, Division of Physics, National Research Laboratories Ottawa.*

It was thought that the phenomenon might be explained by the temperature differential that is created in a large bin of wheat with the onset of winter temperatures. The wheat is warm when the bin is loaded in the early fall, and as wheat is a fairly good insulator the sides, ends, and top of the pile cool much more rapidly than the central bulk of the grain. A large temperature difference is thus established between the inner and outer parts of the wheat pile.

Kiesel *et al.* (1), in the U.S.S.R., have shown that a transfer of moisture takes place from the warmer to the colder portions when a temperature gradient is maintained in wheat. They experimented with grain in 6-litre flasks, through the mouths of which small (20 to 25 ml.) cylindrical cooling coils were inserted. Large increases in the moisture content of the grain around the cooling units were obtained in experiments lasting about 30 days; moulds generally developed and a number of samples germinated.

The experiment described in this paper was made on a larger scale. A temperature differential of 35° C. was established across 6 ft. of grain having an initial moisture content of 14.6%. At the end of the experiment, which lasted 316 days, the maximum moisture content recorded at the cold end (0° C.) was 29.6%, and the minimum recorded at the warm end (35° C.) was 10.9%.

Equipment

A drawing of the equipment is shown in Fig. 1. It consisted of a large insulated box with a metal tank at each end and a space between the tanks, 6 ft. long by 2 ft. wide by 20 in. high, to hold the wheat. The wheat was

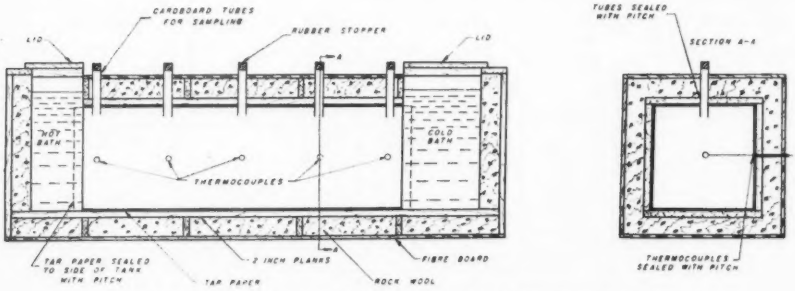

FIG. 1. *Cross section drawings showing side and end elevations of equipment.*

enclosed, top, sides, and bottom, in heavy tar paper, and rested directly against the metal tank at each end. The join in the paper and the joints between the paper and the metal were made tight with pitch. Support was obtained by means of a wooden box made of two-by-fours on edge; and additional insulation consisted of rock wool $3\frac{1}{2}$ in. thick and of fibre board $\frac{1}{4}$ in. thick. Five tubular openings were provided in the top of the box for withdrawing samples. These were located as follows: No. 1, four inches from warm tank;

No. 5, four inches from cold tank; and Nos. 2, 3 and 4, at equidistant intervals (16 in.) between Nos. 1 and 5. These openings were made with wax-impregnated cardboard mailing tubes; the joints between them and the tar paper were sealed with pitch; and rubber stoppers were used to close them. A small brass probe with a capacity of 25 gm. was used for withdrawing samples. Five thermocouples were installed on the central longitudinal axis of the box, one below each of the openings. The cold bath was kept at 0° C. by means of ice, and the warm bath was maintained at 35° C. with a thermostatically controlled heater.

Procedure

The box was filled on February 25, 1942, with about 19 bu. of clean No. 1 Northern wheat that had been thoroughly mixed in a McLelland mixer. The wheat was packed as tightly as possible to prevent settling. After the equipment had been sealed, it was allowed to stand until March 9. At that time the wheat had a moisture content of 14.6% and its temperature was 20° C. Temperatures of 0° and 35° C. were then established and maintained in the cold and warm tanks, respectively. Temperatures were taken periodically with the thermocouples; and at the same time small samples of the wheat were withdrawn from near the central axis of the box through the five sampling holes. Moisture determinations were made by the standard vacuum oven method. The results obtained from this method are, on the average, a fraction of a per cent higher than those obtained with the Brown-Duvel method, which is the official method used in grading grain in Canada. The official moisture content of the wheat at the beginning of the experiment was actually 14.3% (Brown-Duvel) rather than 14.6% as shown by the vacuum oven method. It would therefore have graded "straight", not "tough".

The experiment was stopped on January 19, 1943. The box was opened and 140 samples were taken for moisture tests from points distributed systematically throughout the grain.

Results

Temperature and Moisture Changes During Experiment

The changes in the temperature of the wheat that took place during the experiment are shown in Fig. 2. Five curves are given, one for each thermocouple. The curves show that a temperature equilibrium was established throughout the grain in about 20 days. At that time Thermocouple 1 was reading 28° C. and Thermocouple 5, 9° C.; a difference of 19° C. thus existed between the two points. The remaining three thermocouples gave intermediate readings. The temperature difference between the various points remained relatively constant throughout the course of the experiment. How-

ever, all temperatures were raised about 3° for a considerable period because of hot weather when the warm bath operated erratically and at a higher

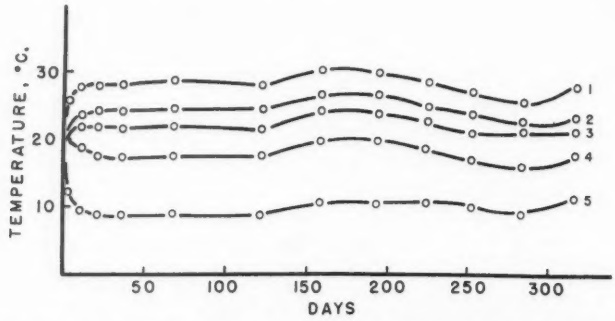

FIG. 2. *Curves showing progressive changes in the temperature of the wheat at five points on the central axis of the box. Curve (1): four inches from warm end; (2): 20 in. from warm end; (3): centre; (4): 20 in. from cold end; and (5): four inches from cold end.*

temperature. This digression from the planned course of the experiment is of little significance and can be disregarded.

It will be observed that the temperatures at the five points are not uniformly distributed. This point is illustrated in Fig. 3, which shows the temperatures on the 36th day. The temperature gradient is not uniform from end to end of the box, and the temperatures may be represented by a sigmoid shaped

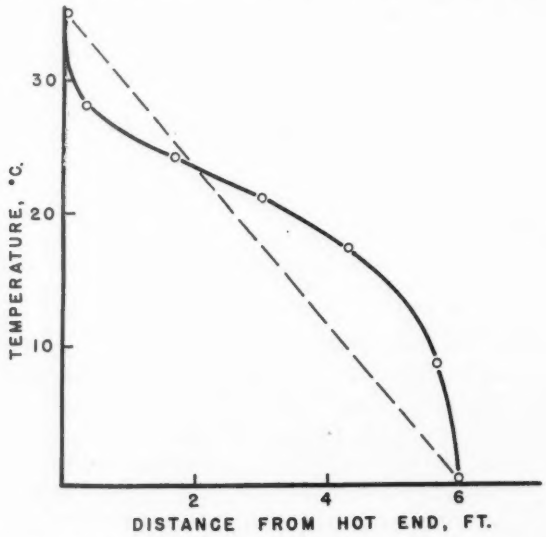

FIG. 3. *Curve showing temperature along central axis on the 36th day.*

curve. The dotted line in Fig. 3 represents a linear gradient and it should be noted that the sigmoid curve is not equally spaced about this line. Comment on this matter can conveniently be reserved until the data on moisture content have been presented.

The changes that occurred in the moisture content of the grain are illustrated in Fig. 4. Only three curves are shown, those for Points 1 and 5, and a curve representing the average moisture content for Points 2, 3, and 4. The curves

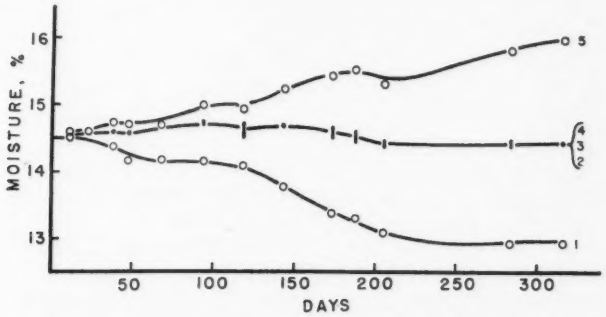

FIG. 4. *Curves showing progressive changes in the moisture content of the wheat at five points on the central axis of the box. Curve (1), four inches from warm end; (2, 3, 4), central positions at which little change in moisture content occurred; and (5), four inches from cold end.*

for the last three points lay so close together that it was impractical to show them separately. However, the range in moisture content found for the three points is indicated by the height of the vertical lines shown in the graph. It is apparent that the range rarely exceeded the experimental errors in sampling and in the moisture determinations. The irregularities in the curves appear to be considerable because of the scale on which they are drawn. They are not much greater than the experimental error involved in withdrawing small (25 gm.) samples and determining the moisture content of them at widely separated intervals; the writers believe that they can be disregarded.

The graph indicates that the moisture content of the grain near the warm end decreased steadily, that the moisture content of the grain at the cold end increased steadily, and that little change in moisture content occurred in the central bulk of the grain. Transfer of moisture from warm to cold wheat took place quite slowly: after 100 days a spread of 0.8% had been created; at 200 days the spread was 2.2%; and at 316 days it was 3.1%. There is no indication that a spread in moisture content would have been created between Points 2 and 4 had the experiment been continued longer. But it is clear that a state of equilibrium had not been reached as the moisture content at the cool end had increased and that at the warm end had dropped very slightly during the last 30 days of the experiment.

Final Condition of the Wheat

The experiment was stopped at the end of 316 days. The top of the box was then removed and 35 samples were taken from the top 2 in. of the grain, at intervals of one foot lengthwise and six inches crosswise. The whole of the top 6 in. layer of wheat was then removed and a second set of samples was taken; and the process was repeated at depths of 12 and 18 in.

The driest sample taken from the warm end had a moisture content of 10.9%, and the wettest sample taken from the cold end had a moisture content of 29.6%. The data, as a whole, are summarized in Table I. In the upper half, labelled top view, the mean is given for the values from the four samples in a vertical row taken one below each other. The lower half of the table, labelled side view, gives the mean of the values from the five samples in the same horizontal line across the box. Thus the two parts of the table give the picture of the moisture conditions looking down on the box and looking at it from the side, respectively. In considering the data, it should be borne in mind that all the wheat had a moisture content of 14.6% at the beginning of the experiment.

TABLE I

MEAN VALUES FOR MOISTURE CONTENT OF WHEAT AT END OF EXPERIMENT

	Distance from hot end, ft.						
	0	1	2	3	4	5	6
Top view							
Left side	11.6	14.3	14.4	14.0	13.7	14.0	18.3
Left middle	11.6	14.2	14.6	14.3	14.3	14.4	19.4
Middle	11.9	14.2	14.3	14.2	14.1	15.0	19.7
Right middle	12.0	14.3	14.4	14.4	14.1	15.4	19.6
Right side	12.4	14.8	14.6	14.4	14.6	15.6	21.8
Side view							
Top 2 in. layer	12.0	14.0	14.2	14.1	14.7	16.2	23.0
Layer 6 to 8 in. down	11.4	14.3	14.5	14.4	14.5	15.2	21.2
Layer 12 to 14 in. down	12.0	14.7	14.5	14.4	14.1	14.4	18.0
Bottom 2 in. layer	12.0	14.6	14.6	14.1	13.5	13.3	16.8

Both top and side views show that the grain next to the warm tank has lost about 2.5% of moisture, but that little change in moisture content has taken place one foot from the warm end, or in the bulk of the grain in the centre part of the box. Next to the cold tank, the moisture content of the grain has increased by 2.2 to 8.4%, and there is evidence of a slight increase in moisture content in certain places at a distance of one foot from the cold tank. It appears that the wheat at the left side of the box was drier

than that at the right side, and that, at the cold end, the wheat at the top was wetter than that at the bottom. These measurements confirm those collected during the course of the experiment and illustrated in Fig. 4; and they show, once again, that the wheat at the warm end lost and that at the cold end gained moisture, but that little change took place in the bulk of the wheat in the central portion of the box.

The data have also been summarized in another way. A model was prepared to represent that part of the grain in which the moisture content changed very little; for this purpose, a moisture range of 14.0 to 15.0% was chosen. The model was then enclosed in a wire cage representing the inside dimensions of the box. Photographs of the model are shown in Fig. 5. At the warm end an almost rectangular block of wheat lost moisture, but at the cold end the contours of the wheat that gained moisture are irregular; they stretch towards the middle of the box at the top, particularly on the right-hand side, and they are close to the end at the bottom, particularly on the left-hand side. The tunnel on the bottom, towards the cold end, also represents wheat that was slightly under 14% in moisture content. The dimple on the right-hand side, towards the warm end, represents wheat of slightly over 15% moisture. Measurements show that the block of wheat in which little change in moisture occurred represented about 60% of the total volume; the drier wheat, including the tunnel (8.5%), represented 24%; and the moister wheat represented 16%. So far as could be determined, the data indicated a negligible loss of moisture from the wheat as a whole.

Discussion

In discussing the significance of these measurements, it is essential first of all to explain the sigmoid shape of the temperature gradient. A curve of this shape indicates a lateral exchange of heat with the exterior; for if the heat flow from the warm end of the box to the cold end had occurred in straight lines, a linear temperature gradient must have been obtained. A consideration of the dimensions and thermal arrangement of the box shows that an exchange of heat with the exterior would take place even when allowance is made for the low thermal conductivity of the rock wool, since the length of the box is considerably greater than either of its lateral dimensions. The divergence of the lines of heat flow in the centre of the box would result in a decreased temperature gradient in that region, as was found experimentally. A curve unequally spaced on the two sides of the linear gradient is also to be expected, since the room temperature was considerably above 17.5° C.—the mid-point between 0° C. and 35° C.—throughout the whole of the experiment.

The experimental work has shown that a temperature gradient was established fairly quickly during the test and maintained during the course of the experiment. The measurements of moisture content show that the wheat at the warm end of the box lost moisture and that at the cold end gained moisture. In order to understand these changes it is necessary to study how the changes of moisture content have been established by the temperature differences.

It may be assumed that at the beginning of the experiment when the wheat was at a uniform temperature and moisture content, the vapour pressure was uniform throughout the box and that this pressure was the equilibrium vapour pressure corresponding to the moisture content of the wheat. As soon as the temperature gradient was established, this equilibrium was upset; at the warm end the relative humidity of the air decreased and the wheat started to lose moisture, while at the cold end the relative humidity increased and the wheat started to gain moisture.

Two principal processes occur in the box: diffusion of water vapour through the air, and interchange of moisture between air and wheat. Diffusion tends to equalize the vapour pressure throughout the air space, thus establishing a humidity gradient governed wholly by the temperature gradient. This stable condition would be rapidly created if the wheat were inert and no interchange of moisture could take place between it and the air. But the wheat is not inert; it gives up or absorbs moisture in an effort to attain an equilibrium between the relative humidity of the air and its own moisture content. Thus, especially at the beginning of the experiment, the wheat tends to equalize the relative humidity throughout the box. However, as there is a temperature gradient in the box, there cannot be both a uniform vapour pressure and a uniform relative humidity; and a dynamic balance must therefore be created between the tendency of diffusion to equalize vapour pressure and the interference caused by interchange of moisture between air and wheat. The comparative ease with which diffusion and interchange take place determines the conditions existing in the box. If the moisture is given off or absorbed by the wheat more quickly than it can diffuse through the box, the relative humidity of the air in any part of the box will be governed primarily by the moisture content of the wheat at that point, and the movement of water vapour will lag behind. On the other hand, if the water vapour diffuses through the air much more rapidly than it can enter or leave the wheat, an almost uniform vapour pressure will be established throughout the box, the relative humidity will be governed by the temperature, and the changes in the moisture content of the wheat will lag. In the final equilibrium a uniform vapour pressure must exist, because the capacity of the wheat to give off and absorb moisture is essentially finite. Under these conditions the temperature will govern the relative humidity, and this in turn will govern the moisture content of the wheat.

It is apparent that in this experiment this final equilibrium has not been approached. There can be no question of a constant vapour pressure throughout the box since the temperature difference between the two ends is so great that in the final equilibrium almost all the moisture would be accumulated at the cold end. From this it can only be assumed that the exchange of moisture between the wheat and the surrounding atmosphere takes place more readily than the diffusion of water vapour from place to place within the wheat. The principal factor governing the conditions in the box is the rate of transmission

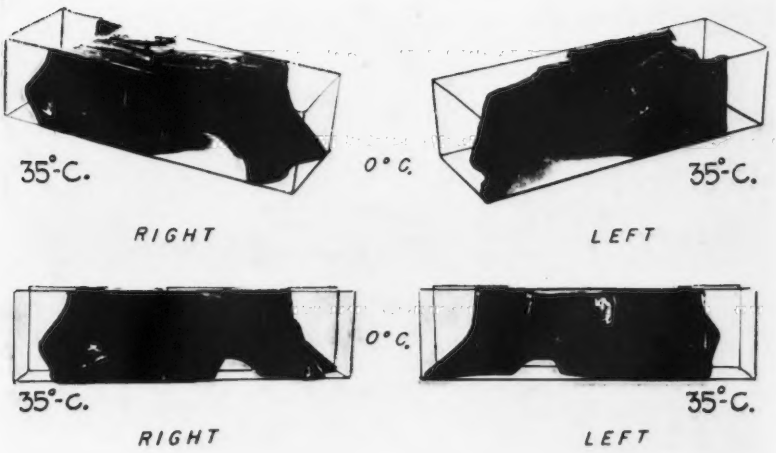

FIG. 5. *Photographs showing four views of model representing grain in which the moisture content remained between 14 and 15%. The wire cage represents the inside dimensions of the box.*

of moisture from one end of the box to the other, rather than the time required for the exchange of moisture between the wheat and the surrounding atmosphere.

A few figures will serve to indicate why this should be so. The weight of a cubic foot of wheat is about 40 lb., and 0.4 lb. of water would be required to raise its moisture content from 14 to 15%. Saturated air at 20° C. contains 0.0011 lb. of water per cu. ft., so that, in order to raise the moisture content of 1 cu. ft. of wheat by 1%, all the water vapour must be extracted from nearly 400 cu. ft. of air. Since in a cu. ft. of wheat the volume of air is less than half, it follows that over 1000 changes of air are required to increase the moisture content by 1%. Thus, even if the air changed once per hour, over 40 days would be required to cause this comparatively small change. As this estimate assumes that all the moisture is extracted from the air by the wheat, it is extremely conservative; but it is adequate to show why the diffusion is the dominant process. Under the conditions existing in a mass of wheat, the time required for moisture to diffuse through the wheat in sufficient quantities to raise the moisture content by an appreciable amount is considerable. This explains why in our experiment the conditions at the end of 316 days were still so far from equilibrium.

In the above discussion it has been assumed that the movement of water vapour was by diffusion from points of high to points of low vapour pressure. The vapour might equally well be transferred by the convective movement of the air in the box and the final moisture distribution indicates that this has occurred. Thus, the fact that very little change has taken place in the moisture content of the wheat in the central part of the box indicates that the moisture movement has occurred through a convection current flowing up the warm end, along the top, down the cold end and back along the bottom. A convection current will also explain nicely why the region of high moisture content at the cold end extends a greater distance towards the centre of the box at the top than at the bottom (cf. Fig. 5). However, whether the movement of moisture takes place by the convective movement of the air as a whole, or by a diffusion of water vapour from a point of high partial vapour pressure to one of low, is immaterial to our argument. One must conclude from this experiment that the movement of water vapour from one place to another within a mass of wheat is a relatively slow process compared with the exchange of moisture between the wheat kernels and atmosphere in the immediate vicinity.

The points brought out by the experiment appear to be as follows: (i) that differences in moisture content can be established in wheat originally uniform by the maintenance of a temperature gradient; (ii) that the establishment of equilibrium under these conditions is a very long process, and is unattainable for any practical length of time or mass of wheat; and (iii) that no uniform moisture gradient is built up between cold and hot portions but instead

discrete areas of high and low moisture are established in the wheat, presumably through transfer of moisture by convection currents created in the interkernel air.

Practical Applications

If the hypothesis of the transfer of moisture from warm to cold grain by convection currents is accepted, it is interesting to apply it to conditions that occur in practice. In Canada, increases in moisture were observed in layers at or near the surface of dry grain in country elevator annexes. They were generally discovered in the spring when attention was drawn to them by heavy infestations of mites.

The annexes were filled in the fall with grain that probably had a temperature of about 70° F. As the weather became colder the surface grain and that near the walls gradually cooled; and it seems reasonable to suppose that this created slow convection currents, which caused the air to flow down in the cold grain just inside the walls and to rise in the centre of the annex. At the time, two years ago, most of the annexes were new and were kept tightly closed so that the conditions were similar to those of the experimental box. The warm air, rising slowly in the centre of the bin, picked up a little moisture—possibly the "sweating" believed to take place in newly-harvested grain played a part—and then deposited it as it passed into the cold grain at the top of the bin. It seems reasonable to assume that this process would slow up and even stop as the winter progressed and the grain became more uniform in temperature. In any event, the increase in moisture content might well escape notice until the grain began to heat with the arrival of warmer spring weather. At that time, rising temperature would also create a rapid increase in the mite population, which would add to the damage.

Reference

1. KIESEL, A., VASSILIEVA, N., and TSYGANKOVA, G. Compt. rend. acad. sci. U.R.S.S. (n.s.) 24 : 786-790. 1939.

EFFECTS OF VARIETY AND ENVIRONMENT ON THE STARCH CONTENT OF WHEAT AND BARLEY[1]

By A. G. McCalla[2] and W. G. Corns[3]

Abstract

Starch content of six varieties each of wheat and barley grown under a wide range of environmental conditions was determined polarimetrically. Environment had a greater effect on starch content than did variety, but both effects were highly significant. Correlation coefficients as high as $-.970$ were obtained between starch and protein content, and for every variety coefficients were highly significant. Regression of starch on protein yielded coefficients numerically well above -1.0 for both wheat and barley.

Barley yielded 16% more starch per acre, on the average, than did wheat. Yields of starch per acre were highest in grain grown on parkland soils, and lowest in that on prairie soils.

Introduction

Many of the industrial uses to which grain might be put are largely associated with the fact that such crops contain relatively large proportions of starch. Few data on the starch content of wheat samples are available, however, and little is known regarding the factors determining starch content. Furthermore, little is known about the relative starch-yielding ability of different crops, or concerning the areas in Western Canada that produce grain of greatest value for industrial purposes.

Most of the earlier pertinent data on wheat starch were summarized by Hopkins and Graham (7). They determined the starch content of average grades (from No. 1 Northern to Feed Wheat) of hard red spring Canadian wheat. Very small and irregular differences were obtained from grade to grade, and starch content did not seem to be closely associated with protein content. Data secured by Hopkins (6), Herd and Kent-Jones (5), and Jacobs and Rask (8) were summarized. These showed extreme variation in starch content from 50.0 to 59.1% on the basis of 13.5% moisture.

The starch content of average grades of recently grown Western Canadian wheat has been extensively investigated by Eva *et al.* (3). The samples represented the principal grades passing through each of the main inspection offices in Western Canada. The mean starch content was 52.5% on a 13.5% moisture basis, the range being from 48.2 to 56.2%. The Garnet grades

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[3] *Graduate Assistant.*

and No. 5 wheat were high in starch. Little difference was found in the results for Grades 1 to 4 Northern. The starch level of all grades at Edmonton was consistently above the average.

Starch determinations on barley have been largely confined to results obtained in connection with malting studies. Ayre, Sallans, and Anderson (1) determined starch in a number of barley varieties grown at stations across Canada. When their results are expressed on a 13.5% moisture basis (used throughout this paper) the station averages are shown to have varied from 44.7 to 51.2% starch. Variation due to varieties was less, the extremes for variety averages being 45.3 and 49.6%. Interstation correlations for protein content and starch content were negative and highly significant, but inter-variety correlations, although also negative, were not significant.

A comparison of yields of starch from barley and wheat grown under comparable conditions is given in the results of yield tests carried out in Saskatchewan (10). In nearly all parts of the province barley outyielded wheat and oats even after allowances were made for the hulls of oats and barley. It seems safe to conclude, therefore, that this Saskatchewan-grown barley produced more starch per acre than did the wheat.

The present work was undertaken in an effort to determine the effect of variety and environment on the starch content of wheat and barley, and to compare these two crops as potential sources of starch for industrial purposes. The results of the studies on flour take on added interest since distillers are now using granular flour as a raw material. The original outline called for three years' work. This plan had to be abandoned when more pressing work took up the facilities of the laboratory. The results obtained in the first year's work are so definite in nature, however, that it was deemed advisable to publish them.

Material and Methods

Three series of samples were investigated.

The first was made up of five varieties of wheat grown at Edmonton on black loam soil and at Fallis on a rather infertile gray soil, in each of 1940 and 1941. The 20 samples of wheat were analysed for protein, and duplicate determinations of starch in the wheat samples and in the flours milled from them were carried out.

The second series was made up of six varieties of wheat grown at eight locations in central and northern Alberta and at Scott and Swift Current in Saskatchewan. The Alberta locations were either on black soil (Edmonton and Beaverlodge) or on gray soil (Athabaska, Bon Accord, Fallis, Mellowdale, Sundre, and Warburg). Single starch determinations were made on each sample of wheat and on the flour milled from each sample, while protein determinations were made on the wheat.

The third series was made up of six varieties of barley grown at the eight Alberta stations listed above for the second series.

At all stations except Athabaska the wheat of Series 2 and the barley of Series 3 were produced under comparable conditions. No comparison can be made of the Athabaska results.

The method used in the determination of starch was a slight modification of the revised Mannich-Lenz method described by Hopkins (6). The Lintner-Schwartz method as outlined by Ayre, Sallans, and Anderson (1) was tried but numerous difficulties with wheat samples led to the rejection of this procedure as a routine practice.

The samples were ground first in a burr mill and then in a ball mill for six hours. Although the ground material was scarcely as fine as that recommended by Hopkins it proved to be entirely satisfactory. On the other hand, in early work in which more coarsely ground grain was used the results were quite unsatisfactory chiefly because great difficulty was encountered in filtering.

The modification of the Hopkins procedure noted above was the substitution of washing under suction (2) for the repeated washing and centrifuging with alcohol to remove soluble materials that are laevorotatory and therefore tend to reduce the apparent starch content of the samples. The sample to be washed was placed on No. 42 filter paper in a Buchner funnel and three portions of 20 ml. each of alcohol, made up as described by Hopkins, were poured over the sample. Under moderate suction, the washing was accomplished in about seven to eight minutes. This procedure appeared to be quite satisfactory since the starch values of samples prepared in this way agreed exactly with those obtained using the centrifuging procedure. The samples were also easier to transfer from funnels than from centrifuge tubes.

In other respects the procedure used followed the directions of Hopkins (6). Samples were polarized using a 10 cm. tube, and six readings were taken for each sample. A specific rotation of 200 degrees was assumed for both wheat and barley starch. For barley starch dispersed in calcium chloride solution, this value has not been determined, but its use seems justifiable for the purposes of the present survey.

Experimental Results

Series 1. Wheat

The analytical data for Series 1 are presented in Table I, while the results of the analysis of variance of these data are given in Table II. These results show the definite effects of both variety and environment on protein and starch content, but show also that environment is much the more important factor. This, of course, merely confirms many experimental results as far as protein is concerned, but essentially the same effect is shown to obtain with

starch. The differences in starch content of wheat appear to be carried through to the flour. Starch, protein, and moisture made up an average of 93.6% of the total flour weight, and only minor variations occurred among

TABLE I

ANALYTICAL RESULTS, SERIES 1, EXPRESSED ON THE BASIS OF 13.5% MOISTURE

Station	Year	Variety					Station mean
		Marquis	Red Bobs	Regent	Renown	Thatcher	
Protein content of wheat, %							
Edmonton	1940	14.6	13.9	15.5	15.3	14.8	14.8
Fallis	1940	11.0	9.5	11.8	14.1	11.0	11.5
Edmonton	1941	15.6	14.5	16.3	15.7	15.7	15.6
Fallis	1941	11.9	11.0	12.6	12.9	11.8	12.0
Mean		13.3	12.2	14.0	14.5	13.3	13.5
Starch content of wheat, %							
Edmonton	1940	52.7	54.4	52.7	54.0	52.6	53.3
Fallis	1940	54.8	57.7	56.4	55.9	58.6	56.7
Edmonton	1941	51.9	54.8	52.3	51.9	52.6	52.7
Fallis	1941	55.0	57.4	53.9	55.0	55.8	55.4
Mean		53.6	56.1	53.8	54.2	54.9	54.5
Starch content of flour, %							
Edmonton	1940	65.6	67.7	64.9	64.9	67.5	66.1
Fallis	1940	67.9	69.2	70.2	67.3	68.5	68.6
Edmonton	1941	65.0	66.4	64.1	64.5	65.4	65.0
Fallis	1941	67.5	69.2	67.5	67.0	67.7	67.8
Mean		66.5	68.1	66.7	65.9	67.3	66.6

TABLE II

ANALYSIS OF VARIANCE, SERIES 1 (TABLE I)

Variance due to:	D.f.	Mean squares		
		Protein	Starch in wheat	Starch in flour
Variety	4	6.06**	11.10**	5.83**
Test	3	40.75**	29.31**	18.47**
Variety × test	12	0.844**	1.65**	1.28**
Residual	20	0.005	0.321	0.091
Total	39			

** *Significant beyond the 1% point.*

samples. It is quite apparent that a high negative correlation exists between protein content and starch content, but since Series 2 and 3 both offer better data for correlation studies, such studies of the data from Series 1 were not made. The results show that residual variance (or laboratory error) is very low in comparison with the interaction, so only single determinations were made on the material in Series 2 and 3.

SERIES 2. WHEAT

The analytical results for Series 2 are given in Table III, and the results of analysis of variance of these data in Table IV. These results confirm in every way those obtained for Series 1, but since the range of conditions under

TABLE III

ANALYTICAL RESULTS, SERIES 2, EXPRESSED ON THE BASIS OF 13.5% MOISTURE

Station	Variety						Station mean
	Renown	Red Bobs	Thatcher	Marquis	Apex	Regent	
Protein in wheat, %							
Sundre	10.5	8.6	10.1	10.4	10.3	10.5	10.1
Mellowdale	11.8	10.6	11.0	10.2	11.0	11.1	11.0
Fallis	13.0	11.8	12.3	11.6	12.2	13.1	12.3
Bon Accord	13.2	11.9	12.1	12.4	12.8	13.4	12.6
Warburg	14.7	13.1	14.9	12.7	14.8	14.7	14.2
Athabaska	14.9	13.6	14.0	15.1	14.5	15.1	14.5
Edmonton	15.5	15.0	15.6	15.2	15.4	15.5	15.4
Swift Current	15.9*	14.5	15.8	15.1	14.8	16.0	15.4
Beaverlodge	16.7	15.4	15.5	15.5	16.3	16.6	16.0
Scott	16.4*	15.2	16.6	15.1	15.7	16.2	15.9
Mean	14.3	13.0	13.8	13.3	13.8	14.2	13.7
Starch in wheat, %							
Sundre	59.4	59.1	58.6	56.5	58.6	58.2	58.4
Mellowdale	56.7	57.6	57.2	56.2	57.6	56.2	56.9
Fallis	54.9	54.5	55.8	53.5	54.4	54.9	54.7
Bon Accord	56.0	53.6	55.5	51.2	53.2	52.5	53.7
Warburg	54.1	54.4	53.8	54.2	53.9	52.0	53.7
Athabaska	54.9	53.2	53.8	52.9	52.0	51.0	53.0
Edmonton	52.6	53.0	51.6	52.9	49.0	51.1	51.7
Swift Current	51.8*	52.2	50.7	51.2	50.3	48.7	50.8
Beaverlodge	51.2	50.2	51.5	50.7	49.5	47.9	50.2
Scott	51.1*	51.4	48.2	50.4	50.5	49.3	50.1
Mean	54.3	53.9	53.7	53.0	52.9	52.2	53.3
Starch in flour, %							
Sundre	70.3	72.1	68.3	68.9	68.2	68.4	69.4
Mellowdale	69.0	68.4	67.9	68.0	66.8	68.4	68.1
Fallis	66.7	67.6	66.3	66.6	65.7	66.8	66.6
Bon Accord	67.8	67.6	66.4	67.0	65.5	65.7	66.7
Warburg	65.5	67.6	65.2	65.1	63.8	65.4	65.5
Athabaska	65.0	65.6	65.3	63.9	62.5	62.3	64.1
Edmonton	65.7	64.2	63.9	64.8	63.0	64.2	64.3
Swift Current	65.2*	66.2	64.0	64.4	63.8	63.7	64.5
Beaverlodge	62.5	62.6	62.7	61.5	61.2	60.7	61.8
Scott	64.0*	63.9	62.6	63.6	63.2	63.0	63.5
Mean	66.2	66.6	65.3	65.4	64.4	64.9	65.5

* *Values for Renown at Swift Current and Scott calculated (4).*

TABLE IV

ANALYSIS OF VARIANCE, SERIES 2 (TABLE III)

Variance due to:	D.f.	Mean squares		
		Protein	Starch in wheat	Starch in flour
Variety	5	2.27**	6.09**	6.70**
Station	9	22.52**	46.57**	30.79**
Interaction	43	0.216	0.940	0.405
Total	57*			

* *Values for Renown at Swift Current and Scott calculated (4).*
** *Significant beyond the 1% point.*

which the samples were grown was far greater, the variations in protein and starch content are likewise much greater. The extreme range of from 47.9 to 59.4% starch is greater than the range reported in the literature reviewed in connection with this work. The value of 59.1% reported by Hopkins (6) was obtained with soft wheat, while the present values are all for hard wheat. It would appear, therefore, that the conditions under which the wheat is grown is the primary factor in determining starch content.

The differences in starch content of the wheat samples are reflected in the differences in the flour samples. The extreme range for starch in flour was from 60.7 to 72.1%. The average percentage of the total flour weight made up by starch, protein, and water was slightly less than in the samples of Series 1, being 92.7%. Since the method of determining starch does not give absolute values for this substance, this total is open to some question.

The relations between starch content and protein content were investigated. Simple correlation and regression coefficients were determined for: first, each variety; second, station means; and third, the individual data taken altogether, regardless of variety or station. These statistics are presented in Table V. With only six varieties included in the study, intervarietal corre-

TABLE V

CORRELATION AND REGRESSION COEFFICIENTS BETWEEN PROTEIN CONTENT OF WHEAT AND STARCH CONTENT OF WHEAT AND FLOUR, SERIES 2

Variety	D.f.	Wheat		Flour	
		r_{sp}	b_{sp}	r_{sp}	b_{sp}
Apex	8	−.936**	−1.50	−.955**	−0.99
Marquis	8	−.801**	−0.82	−.915**	−0.97
Red Bobs	8	−.943**	−1.14	−.956**	−1.16
Regent	8	−.977**	−1.54	−.928**	−1.12
Renown	6	−.959**	−1.18	−.964**	−1.15
Thatcher	8	−.965**	−1.37	−.959**	−0.85
Station means	8	−.970**	−1.33	−.963**	−1.07
Individual results	56	−.877**	−1.25	−.904**	−1.04
Individual results*	51	−.926**	−1.26	−.940**	−1.03

** *Significant beyond the 1% point.*
* *After eliminating the differences between varieties.*

lations were not very satisfactory, although a value of $-.771$ for the correlation between variety averages for starch and protein was obtained.

The high correlation coefficient for each variety indicates the very close inverse relation between starch and protein contents of wheat. It seems clear that the factors that determine the protein content of a sample go a long way towards determining the amount of starch it will contain, but this does not imply a causal relationship between the two.

Perhaps the regression coefficients are of even more interest, however, for they show that for every decrease of 1% in protein content there was an increase of considerably more than 1% in starch. There was a barely significant difference in the values of the regression coefficients, that for Marquis being significantly lower than those for Apex and Regent. There was no significant difference in the values of the regression coefficients for flour and these were lower than for wheat. This suggested that grain characteristics such as kernel size and bushel weight might be important in determining starch content. Further correlation and regression studies were therefore carried out on the wheat data, introducing these two factors as independent variables. The results are given in Table VI.

TABLE VI

RELATIONS BETWEEN STARCH AND PROTEIN CONTENT OF WHEAT

Statistic	Station means		Individual results	
	D.f.	Value	D.f.	Value
r_{sp}	8	$-.970^{**}$	56	$-.877^{**}$
$r_{sp.b}$	7	$-.953^{**}$	55	$-.874^{**}$
$r_{sp.k}$	7	$-.983^{**}$	55	$-.872^{**}$
$r_{sp.bk}$	6	$-.948^{**}$	54	$-.831^{**}$
r_{sb}	8	$.775^{**}$	56	$.607^{**}$
$r_{sb.p}$	7	$.729^{*}$	55	$.395^{**}$
r_{sk}	8	.364	56	.173
$r_{sk.p}$	7	.595	55	.059
b_{sp}	8	-1.33	56	-1.25
$b_{sp.b}$	7	-1.13	55	-1.05
$b_{sp.k}$	7	-1.26	55	-1.19
$b_{sp.bk}$	6	-1.18	54	-1.05
$R_{s.pb}$	7	$.986^{**}$	55	$.897^{**}$
$R_{s.pk}$	7	$.985^{**}$	55	$.878^{**}$
$R_{s.pbk}$	6	$.987^{**}$	54	$.923^{**}$

NOTE: *s = starch, %; p = protein, %; b = bushel weight, lb.; k = 1000-kernel weight, gm.*
* *Significant beyond the 5% point.*
** *Significant beyond the 1% point.*

Weight per bushel is shown by these results to have had an effect on starch content (accounting for 1.8% difference in starch in the extremes of station means) but weight per 1000 kernels apparently had not. The reduction of the regression coefficient for station averages from -1.33 to -1.13 is statistically significant. The variations in environmental conditions that

favour low protein content favour high weight per bushel, and also affect other constituents of the wheat kernel probably in a more or less regular fashion. If all of these variations could be accounted for, the regression $b_{sp.b\ldots\ldots x}$ should approach -1.0.

The multiple correlation coefficient $R_{s.pb}$ is, statistically, significantly higher than the simple coefficient r_{sp} for both station means and individual results. Only with the latter does the introduction of 1000-kernel weight increase this multiple coefficient, and then only in conjunction with weight per bushel. From the practical point of view, of course, the increases are rather small, but would be of some value if starch were to be predicted from other analytical data.

No partial correlation studies were carried out with data for individual varieties.

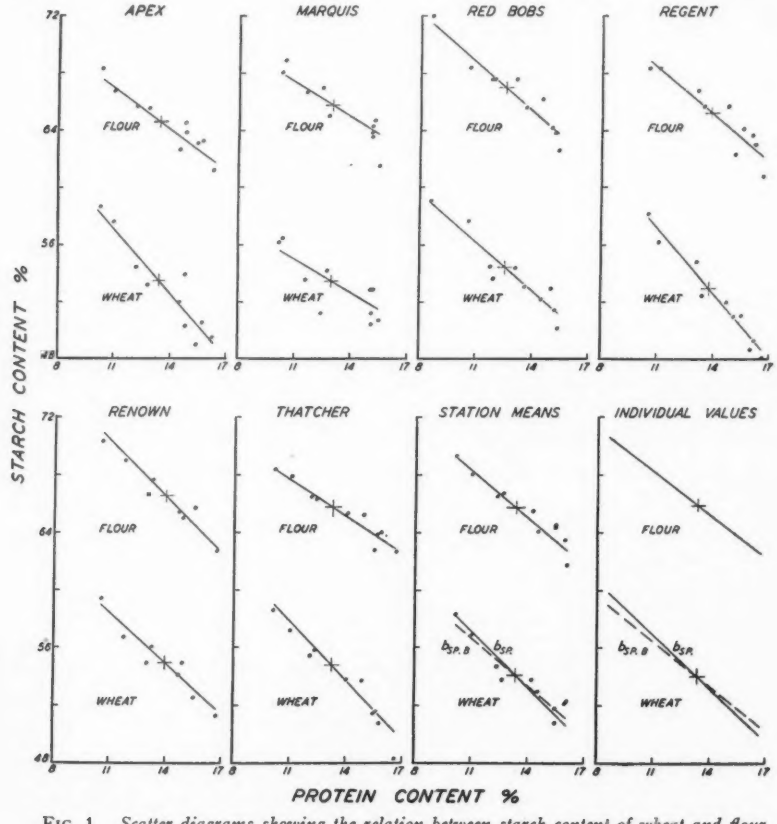

FIG. 1. *Scatter diagrams showing the relation between starch content of wheat and flour and protein content of wheat. The regression lines for individual values were determined after variety differences had been eliminated.*

The value of the regression studies is further seen by an examination of Fig. 1. This shows that there are marked differences in varieties as far as the relation between starch and protein is concerned. The significance of the differences in centroids was determined and yielded an F value of 6.09 with a 1% point of 3.40. The comparable determination for flour yielded an F value of 7.76. Thus the varieties differ significantly in starch content at mean level of protein. This is a true variety effect, since the effect of environment in determining the relationship of starch and protein was essentially the same for the varieties (Marquis excepted). If high starch content of a sample were the most important factor to a processor, then these differences in varieties might be quite important in making a choice of the most suitable variety to grow. It should be noted, however, with this particular series, that some other varieties contain as high a percentage of starch as does Renown since, when grown under comparable conditions, they contain less protein. Thus Renown, despite its highly placed regression line, might not be the best variety for the processor. Weight per bushel accounted for only a small part of the variation in centroids, since Renown was only 0.6 lb. above the mean weight, and the partial regression coefficient, although statistically significant, was only 0.52% per lb.

The differences in starch content of wheat varieties also largely carry through to the flour, as the regression line of Renown is placed significantly higher than are the lines for the other varieties. There are, therefore, other undetermined factors affecting the varietal relation between starch and protein. It seems likely that bran thickness, or percentage bran, may be an important factor but, while this factor is possibly related to bushel weight, there was no direct means of checking its effect. Flour yields might have been used, but a preliminary examination of the data suggested that a complete study would not be worth the time it would take.

The results for this series show, therefore, that both environment and variety affect the starch content of wheat, but environment has considerably more effect than variety. There is a highly significant negative correlation between protein content and starch content, and the regression of starch on protein is, numerically, considerably greater than -1.0. Allowing for weight per bushel reduces the regression coefficient significantly, but this is only one of probably many factors that affect the value of this coefficient. The starch content of flour is also highly correlated with protein content of wheat, while the regression coefficients are much lower than for wheat, but are numerically slightly above -1.0 on the average. There are statistically significant differences in the position of the regression lines for individual varieties of wheat and these differences are largely maintained with the flour.

Series 3. Barley

The analytical results for Series 3 are presented in Table VII and the results of analysis of variance of these data in Table VIII.

TABLE VII

ANALYTICAL RESULTS, SERIES 3, EXPRESSED ON THE BASIS OF 13.5% MOISTURE

Station	Variety						Station mean
	Trebi	Sel.*	Olli	Regal	*O.A.C.* 21	Newal	
Protein in barley, %							
Sundre	7.9	6.9	8.3	9.1	7.8	9.0	8.2
Mellowdale	9.2	8.4	9.7	9.7	10.2	10.1	9.6
Fallis	9.4	9.8	11.0	11.0	10.5	11.4	10.5
Bon Accord	10.4	10.2	11.8	11.1	11.2	11.0	11.0
Warburg	13.3	11.5	13.0	13.9	12.8	14.4	13.2
Athabaska	12.0	11.4	12.4	11.6	12.2	13.6	12.2
Beaverlodge	13.2	13.7	13.9	15.0	14.6	15.7	14.4
Edmonton	14.0	14.0	13.1	13.3	14.0	14.0	13.7
Mean	11.2	10.7	11.7	11.8	11.7	12.4	11.6
Starch in barley, %							
Sundre	52.2	54.6	54.2	50.6	50.7	51.3	52.3
Mellowdale	52.3	52.4	51.5	50.7	49.7	51.0	51.3
Fallis	53.4	51.9	50.3	48.8	48.6	47.8	50.1
Bon Accord	50.5	49.9	49.3	48.2	47.1	46.9	48.7
Warburg	48.1	48.9	49.0	48.1	46.5	44.8	47.6
Athabaska	48.8	47.9	48.3	47.4	44.8	43.5	46.8
Beaverlodge	48.0	48.1	47.4	44.1	44.5	42.1	45.7
Edmonton	47.0	43.6	47.4	45.4	43.4	46.3	45.5
Mean	50.0	49.7	49.7	47.9	47.0	46.7	48.5

* *A rough-awn selection from Newal.*

TABLE VIII

ANALYSIS OF VARIANCE, DATA FOR SERIES 3 (TABLE VII)

Variance due to:	D.f.	Mean squares	
		Protein	Starch
Variety	5	2.60**	17.85**
Station	7	27.82**	38.65**
Interaction	35	0.300	1.35
Total	47		

** *Significant beyond the 1% point.*

The results show that the situation with barley is comparable to that with wheat. Both variety and environment affect protein and starch content, but environment is the more potent factor. The range in starch content of from 42.1 to 54.6 was slightly more than with individual wheat samples, while the average starch content was only 5.6% below that of the wheat grown at the same stations.

As in wheat, the starch content was significantly correlated with protein content. Simple correlation and regression coefficients are presented in Table IX. The correlation coefficients are of the same order of magnitude as

TABLE IX

CORRELATION AND REGRESSION COEFFICIENTS BETWEEN STARCH AND PROTEIN CONTENT OF BARLEY, SERIES 3

Variety	D.f.	r_{ap}	b_{ap}
Newal	6	−.928**	−1.30
O.A.C. 21	6	−.955**	−1.13
Olli	6	−.970**	−1.18
Regal	6	−.889**	−0.99
Trebi	6	−.946**	−1.00
Selection	6	−.930**	−1.29
Station means	6	−.963**	−1.13
Individual results	46	−.881**	−1.20
Individual results*	41	−.926**	−1.15

** *Significant beyond the 1% point.*
* *After eliminating differences between varieties.*

those found in wheat, while the regression coefficients are a little lower. Partial correlation and regression coefficients between starch and protein, independent of the effect of weight per bushel, were determined, but there were no alterations in the regression coefficient for either station means or individual results. No data concerning 1000-kernel weight were available as the samples had been discarded before the statistical calculations were carried out. From the work of Ayre, Sallans, and Anderson (1) it seems likely that the introduction of this factor might have had a marked effect, since they found starch content to be significantly correlated with 1000-kernel weight quite independently of the effect of protein.

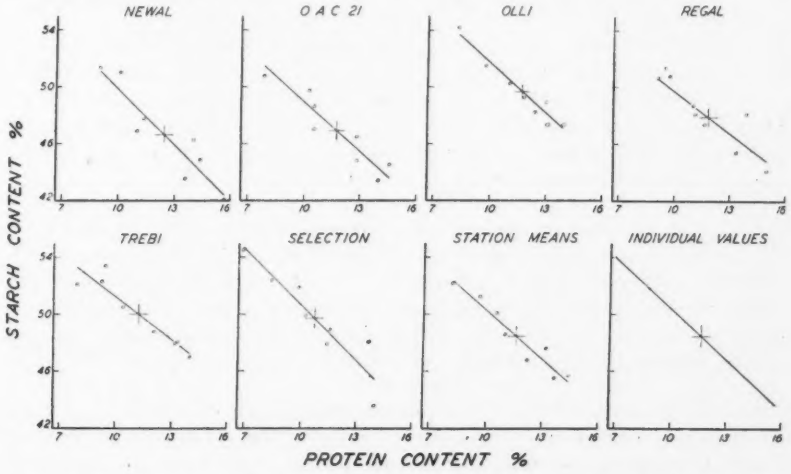

FIG. 2. *Scatter diagrams showing the relation of starch content and protein content of barley. The regression lines for individual values were determined after variety differences had been eliminated.*

The regression lines plotted in Fig. 2 show a variability comparable to that found with wheat, since the F value for significance of difference in centroids is 8.30 with a 1% point of 3.50. There were no significant differences in the regression coefficients, however. Trebi and Olli were outstanding in starch content in relation to protein, and these two offer a marked contrast in physical characteristics. Trebi is a coarse, large-seeded, but rather low-bushel-weight variety, while Olli is a thin-hulled, small-seeded, high-bushel-weight variety. It seems likely, therefore, that Olli yields a highly placed regression line because of its thin hull and high bushel weight, while Trebi yields a similar line because of its large kernels. These observations lead to the conclusion that there are a number of factors that affect the relationship between starch content and protein content, and therefore modify the correlation and regression coefficients.

The results with this series show, therefore, that barley behaves much the same as wheat in relation to variations in starch content of different samples. Environment has a greater effect than variety in determining starch content, but both are highly significant. Highly significant negative correlations exist between starch and protein content, and the regression coefficients are, on the average, numerically well above -1.0. The introduction of weight per bushel as an independent variable does not alter these statistics, but it is believed that if the effects of both this factor and 1000-kernel weight could have been studied simultaneously, they would have been significant. There are significant differences in the position of the regression lines for individual varieties.

Yield per Acre of Starch from Wheat and Barley

At all stations in Alberta except Athabaska the wheat and barley samples were grown side by side under comparable conditions. This makes it possible to compare the acre yields of starch from each variety of wheat and barley. These results are presented in Table X. No detailed discussion of individual results is necessary, but several very interesting conclusions emerge from a consideration of the table as a whole.

Barley yielded more starch per acre than wheat, the average difference for all stations and varieties being 114 lb. of starch per acre, or 16% more starch from barley than from wheat. Thus, in a program involving the use of cereals to supply starch for industrial purposes, barley may quite conceivably be a better source than wheat. At present, wheat is in surplus supply but this need not determine future trends.

There is some question as to the practical importance of yield per acre from the industrial point of view, since the processor is concerned with yields from a unit of material, not with yields from an acre. It seems quite possible, however, that better material for processing may come from areas where

TABLE X

COMPARATIVE YIELDS OF STARCH FROM WHEAT AND BARLEY, SERIES 2 AND 3, POUNDS PER ACRE

Station	Regent wheat	Apex wheat	Renown wheat	*O.A.C.* 21 barley	Marquis wheat	Olli barley	Thatcher wheat
Beaverlodge	866	1021	823	986	1216	1065	1124
Edmonton	718	640	792	853	854	937	710
Bon Accord	652	648	846	774	716	644	853
Mellowdale	655	747	827	637	553	682	720
Fallis	534	604	620	654	577	652	763
Warburg	599	653	562	713	833	776	736
Sundre	409	429	492	418	403	435	465
Mean	633	681	709	719	736	742	767

Station	Red Bobs wheat	Regal barley	Newal barley	Trebi barley	Selection barley	Mean yields	
						Wheat	Barley
Beaverlodge	1024	1148	859	1491	1454	1012	1167
Edmonton	764	1081	1242	1048	764	746	988
Bon Accord	718	957	979	1077	1028	739	910
Mellowdale	975	735	951	799	1120	746	820
Fallis	644	792	831	1056	887	624	812
Warburg	836	596	583	637	948	703	709
Sundre	567	343	369	418	555	461	423
Mean	790	806	831	932	965	719	833

starch yields per acre are reasonably good. If this proves to be true, the location of plants in such areas would, in the opinion of the writers, be advantageous from both the producers' and processors' points of view.

Varieties of each crop differed widely in starch yielding ability. Thus, Renown wheat, which contained the most starch in relation to protein content, yielded less starch per acre than Marquis, Thatcher, or Red Bobs wheat, and less than each of the varieties of barley. Newal barley, which contained the least starch in relation to protein content, yielded more starch per acre than any variety of wheat, and more than was obtained from *O.A.C.* 21, Olli, or Regal barley.

The station yields in pounds were, to a considerable extent, the reverse of the station starch values expressed as percentages of the grain. Thus grain grown at Sundre contained more starch per unit of grain than that grown at any other station but produced less starch per acre than any other of the Alberta-grown samples. Grain grown at Beaverlodge, on the other hand, contained nearly the lowest percentage of starch, but produced more per acre than that grown at any other station. These differences are, of course, related to soil fertility and general productivity. The soils at Edmonton and Beaverlodge are much more fertile than those at the other Alberta stations. Undoubtedly the order of stations would be changed in other years, but enough

is known about these stations to say definitely that Edmonton and Beaverlodge would produce the larger yields nearly every year. Of the stations on gray soils, Bon Accord did the best, and this is definitely the best of these soils. The soil at Sundre has proven to be of particularly low fertility, as it usually gives poor yields of grain that is lower in protein than that secured from any other station included in this study.

The yields of starch from wheat grown at Swift Current and Scott, while not presented in Table X, are of interest for purposes of comparison. The highest yield at Swift Current was 429 lb. of starch, a figure almost as low as the lowest obtained for wheat at Sundre, while the average was 367 pounds, 94 lb. lower than the average for wheat at Sundre. Yields at Scott were decidedly lower, the average being 268 lb. per acre, or only 26.5% as much as at Beaverlodge. The locality in which grain is grown, therefore, has an enormous effect on the yield of starch that can be expected.

Discussion

Extended discussion of one year's results is probably not warranted, but these results, taken together with other information available, are so definite in nature that little doubt as to their general validity can be entertained.

From the processing point of view, and particularly with respect to the fermentation industries, the amount of starch per unit of raw material is probably the most important consideration. If the quantity of starch in the grain were the only factor of importance, then plants should be located where the percentage starch is highest. There are, however, undoubtedly many factors other than starch content that will influence the choice of raw material. For example, while barley has a lower starch content than wheat, it may be a more satisfactory material for some industrial purposes. Similar considerations will apply to the choice of area from which the grain is obtained, and perhaps even to the selection of a particular variety if one is outstandingly better than others.

It has been pointed out by Eva *et al.* (3) that judicious selection of individual carlots of wheat at Edmonton, or even at Winnipeg, would probably result in the securing of grain of higher than average starch content for the production of alcohol. It is further stated that it is believed that the advantages to be gained by such selection will more than offset the difficulties involved. If it can be shown, as seems very likely, that certain areas consistently produce grain of high starch content, then it would seem that location of the plants in such areas, plus judicious selection, should insure even better raw material.

The possible importance of yield per acre has already been discussed. Long term averages show that the highest yields of grain are obtained from the black or parkland soils of Western Canada. The results presented here show that starch yields, expressed on the acre basis, are highest for grain grown on these soils, even though the percentage of starch is lower than in

grain grown on the gray or wooded soils. If other factors in the selection of sites for industrial plants were equal, such results as these might have a definite value.

If wheat were to be used for such industrial purposes it would be advantageous from the export point of view if the poorer quality wheat grown in the northern parts of Western Canada in general and in the western part of Alberta could be utilized. A large proportion of the wheat grown in these areas produces bread that is too poor in quality to meet the requirements of either domestic processors or of importers of Canadian wheat (9). The diversion of appreciable quantities of this wheat for industrial uses would serve an excellent purpose in reducing the quantity that normally goes into export channels. Whether such diversion is practicable remains to be seen.

If barley proves to be more satisfactory than wheat for industrial purposes, this crop could replace the poor quality wheat now grown in northern areas, since it can be more easily grown under these conditions and gives larger returns in pounds per acre. It would appear advisable, therefore, to give every consideration to the possible utilization of northern grown cereals for industrial purposes. There may be good reasons why they would not be as satisfactory as some others; but such utilization would in the long run be of benefit to the wheat producers of Western Canada, regardless of their location.

References

1. AYRE, C. A., SALLANS, H. R., and ANDERSON, J. A. Can. J. Research, C, 18 : 169-177. 1940.
2. ETHEREDGE, M. P. J. Assoc. Official Agr. Chem. 24 : 113-119. 1941.
3. EVA, W. J., LEVI, I., and ANDERSON, J. A. Can. J. Research, C, 21 : 173-179. 1943.
4. GOULDEN, C. H. Methods of statistical analysis. John Wiley and Sons, New York. 1939.
5. HERD, C. W. and KENT-JONES, D. W. J. Soc. Chem. Ind. 50 : 15T-22T. 1931.
6. HOPKINS, C. Y. Can. J. Research, 11 : 751-758. 1934
7. HOPKINS, C. Y. and GRAHAM, R. P. Can. J. Research, 12 : 820-824. 1935.
8. JACOBS, B. R. and RASK, O. S. Ind. Eng. Chem. 12 : 899-903. 1920.
9. McCALLA, A. G. and ROSE, D. Univ. Alberta, Coll. Agr., Bull. 37. 1941.
10. SASKATCHEWAN COOPERATIVE WHEAT PRODUCERS LTD. Junior Cooperative Variety Tests, 1942. April, 1943.

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THE CARDIAC ACTION OF POSTERIOR PITUITARY EXTRACT IN PHYSIOLOGICAL DOSES, IN THE NORMAL DOG, AND AFTER PARTIAL AND COMPLETE DENERVATION OF THE HEART[1]

BY MARGARET E. MACK. SAWYER[2] AND G. H. ETTINGER[3]

Abstract

In the normal dog continuous infusion of dilute posterior pituitary extract produces a maximal inhibition of the heart, i.e. slowing to about one-half of the resting rate, with usually a rise in blood pressure of 10 to 30 mm. of mercury.

After bilateral thoracic sympathectomy, posterior pituitary extract also produces maximal inhibition. This inhibition, like that produced in the normal dog, is abolished by atropine.

After bilateral vagotomy posterior pituitary extract produces a moderate but not maximal inhibition. This inhibition is not abolished by atropine.

After bilateral thoracic sympathectomy and unilateral vagotomy, posterior pituitary extract produces a maximal effect.

After total denervation of the heart, posterior pituitary extract produces no inhibition of the heart and the rate is unchanged.

Characteristic changes are produced by posterior pituitary extract in the electrocardiogram of normal dogs. After total denervation no change takes place.

It is concluded that the slowing of the dog's heart that is produced by continuous intravenous infusion of posterior pituitary extract is entirely due to its action through the inhibitory fibres of the vagus and sympathetic nerves.

The bradycardia produced in the dog by posterior pituitary extract, according to Resnik and Geiling (11), and Gruber and Kountz (4), is due to a two-fold action: (*a*) a reflex vagal slowing, and (*b*) a direct depressant action on the myocardium, possibly through coronary arterial constriction. Melville (7) considers that there is no direct myocardial depression; all changes apart from vagal slowing are caused by constriction of the coronary arteries. In the cat, Bacq and Dworkin (1) obtained results that indicated that the slowing produced by pitressin was entirely due to an action upon the extra-cardiac connections of the cardiac nerves.

In order to re-explore the site and nature of the inhibitory process, we determined the action of posterior pituitary extract in the normal dog, and in the dog after partial and complete cardiac denervation. These experiments were done in the course of an investigation of the effects of chronic

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excitation of the inhibitory mechanism of the dog's heart, by infusion of a dilute solution of posterior pituitary extract for two hours daily, over a period of four and one-half years.

Previous investigators tested the action of posterior pituitary extracts, including pitressin, by injecting large single doses of commercial or other concentrated preparations; these doses are more liable to be toxic than physiological. The results were often complicated by the use of anaesthesia. In our experiments a more nearly physiological method of administration was used. Posterior pituitary extract was slowly administered in dilute solution by continuous infusion for 30 to 120 min. to trained, unanaesthetized dogs weighing from 12 to 19 kg., at such a rate as to produce a continuous maximal bradycardia without disturbing effects such as nausea or vomiting. This required only about two to two and one-half pressor units per hour.

Method

Dogs were trained to lie quietly on a table, until their resting-heart rates could be obtained accurately before each infusion was started. After application of alcohol to the leg, a sterile hypodermic needle was inserted into a leg vein and taped in place. This was connected to an outlet on the bottom of an elevated sterile flask containing the infusate by a sterile rubber tube, on the course of which was an air trap by which the rate of flow could be determined in drops. Infusion by gravity flow was continued for 30 to 120 min. and the heart rate counted every 5 to 10 min. Occasionally a continuous count was made for five minutes after the start of the infusion to obtain any immediate changes. After a few trials the dogs remained quiet throughout the infusion, which usually seemed to have a sedative effect.

TABLE I

THE EFFECT OF INFUSION OF POSTERIOR PITUITARY EXTRACT ON THE HEART RATE OF THE DOG, NORMAL, AND WITH CARDIAC SYMPATHECTOMY, REPEATED DAILY OVER A PERIOD OF YEARS

Dog	Condition	Resting rate per minute	Minimal infusion rate, per min.	Resting rate per minute	Minimal infusion rate, per min.
		1938		1942	
Sandy	Normal	64	30	60	44
Toy	Normal	66	40	80	44
Scarface	Normal	68	30	76	36
Rufus	Normal	72	40	88	44
		1940		1942	
General	Cardiac sympathectomy	64	36	72	44
Sargent	Cardiac sympathectomy	68	44	68	40
Ted	Cardiac sympathectomy	72	40	76	44

The pituitary extract used was prepared by the Connaught Laboratories, Toronto, Ont. It contained 10 pressor units per cc., and had tricresol, 0.1% as a preservative. A solution was made of 1 cc. of the extract dissolved in 500 cc. of sterile normal saline. Each animal received as much as 250 cc. of this dilute solution. Daily infusion for periods up to four and one-half years produced little evidence of a tolerance (see Table I).

The effect of atropine (0.2 to 0.3 mg./kg.), administered intravenously before infusion and during infusion, was determined in all dogs after repeated experiments had demonstrated the normal response to posterior pituitary extract.

The effect of the infusion on blood pressure was determined by connecting a mercury manometer to a sterile needle or arterial cannula inserted into the femoral artery under local anaesthesia.

Cardiac denervation was done after the normal response to posterior pituitary infusion had been recorded. The response to infusion and the effect of atropine on the response were determined after operative recovery from each step in the denervation. The sympathetic nerve supply to the heart was interrupted by aseptically removing the stellate ganglia and the thoracic sympathetic chains down to the ninth rib, in two steps, after the method of Cannon *et al.* (2). Cervical vagotomy was done in two stages by aseptic removal of 3 cm. of each of the nerves. At least six days were allowed to elapse between operations. In most cases a period of several weeks passed. Complete denervation of the heart was done in three stages: first, right thoracic sympathectomy and right cervical vagotomy; second, left thoracic sympathectomy; third, left cervical vagotomy. Excision of the vocal cords at the time of the first stage prevented any later difficulty in respiration. Completely vagotomized dogs retained little food, but were maintained for periods of 10 days to two months by supplementing feeding with intravenous infusion of glucose saline.

Electrocardiograms were taken on all animals at all stages of the experiment.

Results

A. Normal Animals

The Effect of Posterior Pituitary Infusion on the Heart Rate

The resting-heart rates in the trained dogs were from 60 to 90 per minute. Continuous infusion with posterior pituitary extract invariably inhibited the heart to about half the resting rate (Table I). This inhibition commenced about one to two minutes after the start of infusion, became maximal in 5 to 10 min. and was maintained thereafter as long as the infusion continued. There was sometimes a very brief period of acceleration in the first minute immediately after the start of infusion, before the heart began to slow. (This

acceleration was always observed by Gruber and Kountz (4) and by Resnik and Geiling (11) who used large single injections of extract.) After the infusion was stopped the heart rate gradually increased and came back to the resting rate in 20 to 30 min. Figs. 1 and 2 show characteristic

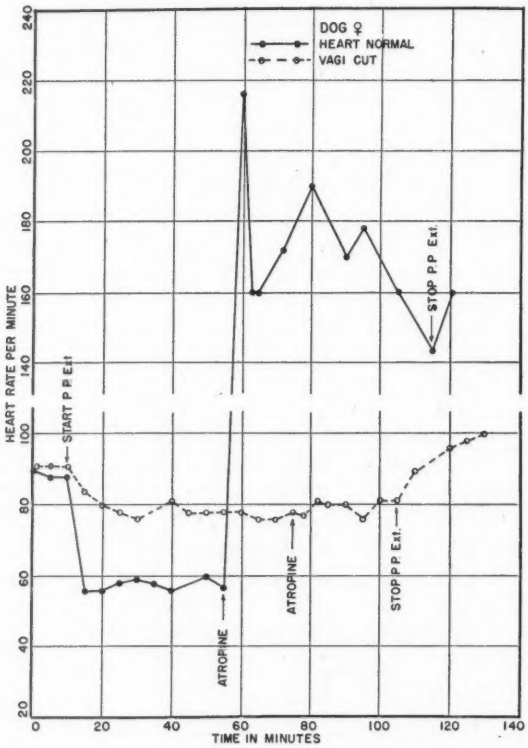

FIG. 1. *The effect of posterior pituitary infusion and the subsequent injection of atropine on the heart rate of a normal, unanaesthetized dog before and after section of the vagi.*

changes in heart rates in normal dogs during infusion with posterior pituitary extract.

The Effect of Atropine on the Response to Posterior Pituitary Infusion

The effect of atropine varied according to whether it was administered before the infusion had started, or during the infusion. Given without infusion it produced a tachycardia up to 200/min. within five minutes, followed by a slow and steady decline in rate, with a return to the normal in one and one-half to two hours. When pituitary extract infusion was started 10 to 30

min. after the atropine injection, it usually produced no inhibition. The heart either maintained the atropine effect with its gradually declining influence, or there was a brief period of further acceleration (Fig. 3). This latter response is similar to that reported by Resnik and Geiling (11) following a

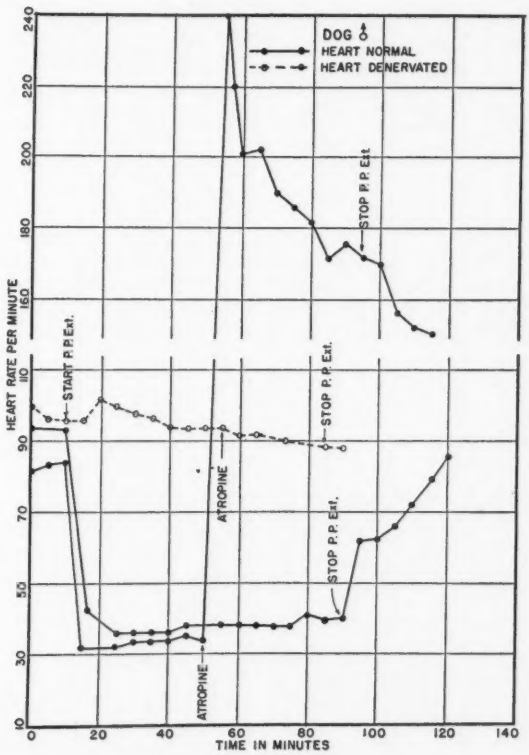

FIG. 2. *The effect of posterior pituitary infusion and the subsequent injection of atropine on the heart rate of a normal, unanaesthetized dog before and after complete denervation of the heart.*

single injection of posterior pituitary extract. Gruber and Kountz (4) found that pitressin slightly slowed the atropinized heart.

When atropine was administered during infusion with posterior pituitary extract, and while the bradycardia was maximal, it invariably produced an intense tachycardia within two minutes (Figs. 1 and 2). The heart rate was higher than that producible in the same dog by atropine alone. For example, in a dog in which infusion of posterior pituitary extract normally slowed the

heart rate from 62 to 34 per minute, and in which atropine without infusion raised the heart rate to 210 per minute, atropine during infusion raised the rate to 244 per minute.

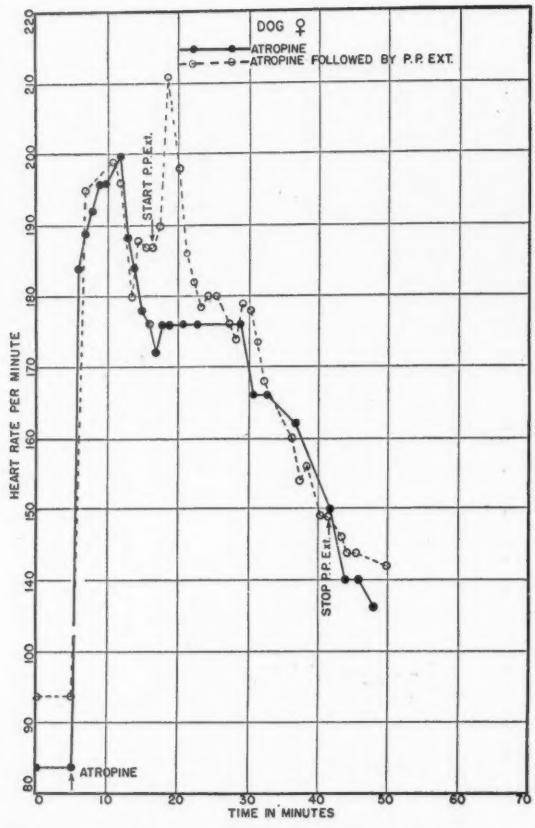

FIG. 3. *The effect of atropine, and atropine followed by infusion of posterior pituitary extract, on the heart rate of a normal, unanaesthetized dog.*

It is clear that atropine prevents the bradycardia produced by posterior pituitary infusion.

Electrocardiographic Changes Produced by Posterior Pituitary Infusion

A number of investigators have studied the effect of single injections of posterior pituitary extract and pitressin on the electrocardiogram. A list of references may be found in a paper by Melville (7). Electrocardiograms taken during continuous infusion showed many changes similar to those reported

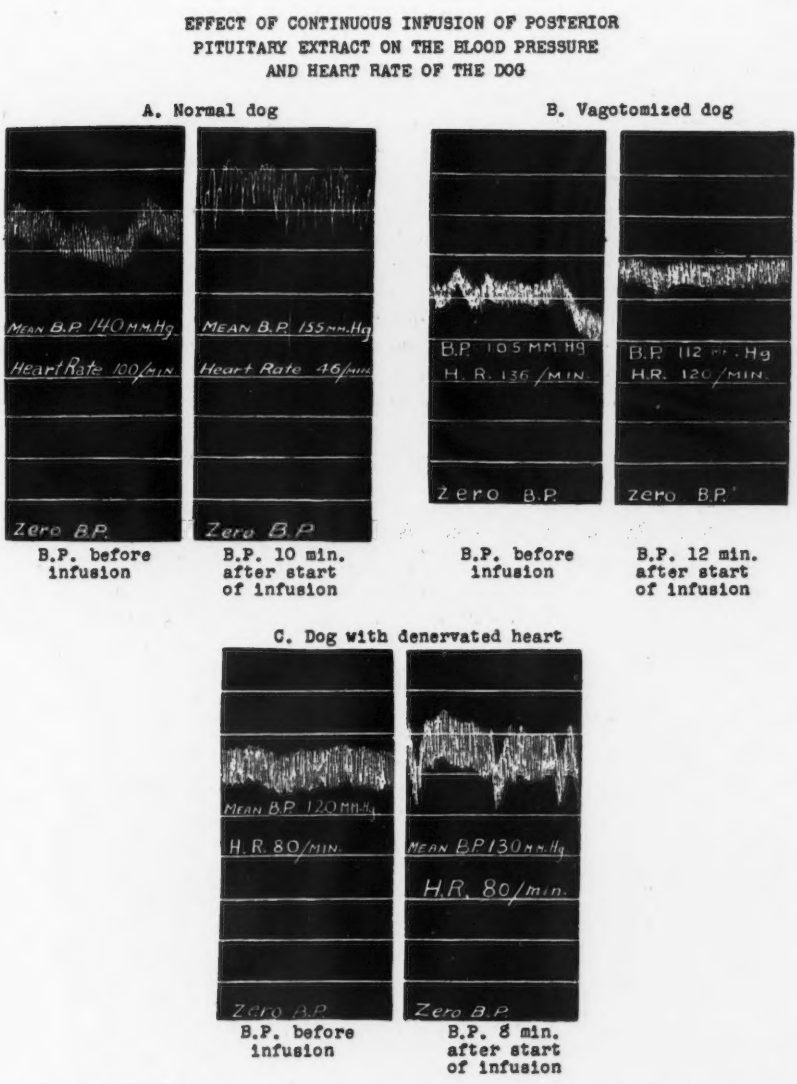

FIG. 4. *Change in femoral arterial blood pressure produced by infusion of posterior pituitary extract under local anaesthesia in the dog, normal, after vagotomy, and after complete denervation of the heart.*

after single injections (Figs. 5 and 6). Within two minutes of the start of infusion a marked slowing in rate took place, sometimes accompanied by an increased *P–R* interval. Changes in the *T* wave were most frequently

observed. Often within 30 sec. of the start of the infusion it became much more prominent, its amplitude increased and its shape peaked. A *T* wave inverted or diphasic before infusion might become upright and more prominent during infusion. Changes in the *P* wave were not common. Occasionally it became slightly notched. Sometimes heart block occurred. Grouping of beats was occasionally seen after infusion had gone on for some time; pulsus bigeminus was the most common of these.

The tachycardia produced in the normal dog by atropine was accompanied by a shortened *P–R* interval, without disturbance of the *T* wave. Infusion of posterior pituitary extract after atropine produced, within one minute, the characteristic heightening of the *T* wave, but no other changes. Atropine injected during the infusion produced a lengthened *P–R* interval, heart block, irregularity with pulsus bigeminus and trigeminus, all within the first half-minute. The *T* wave remained high. After this brief irregularity a marked regular acceleration occurred, with shortened *P–R* interval, the *P* and *T* waves frequently superimposed (Figs. 5 and 6). Gruber and Kountz (4) noted similar effects of atropine given two to three minutes after a single large dose of pitressin.

The Effect of Posterior Pituitary Infusion on Blood Pressure

A single large dose of pitressin or pituitary extract in the unanaesthetized dog produces (3, 4, 6, 7, 14) a sharp fall in blood pressure, preceded by a slight rise and followed by a second rise. A smaller dose may produce a pure

TABLE II

CHANGES IN HEART RATE AND BLOOD PRESSURE PRODUCED IN THE DOG UNDER LOCAL ANAESTHESIA BY INFUSION WITH POSTERIOR PITUITARY EXTRACT

Dog	Condition	*Resting heart rate per min.	Minimal infusion heart rate per min.	Fall in heart rate per min.	Resting blood pressure, mm. Hg.	Infusion blood pressure, mm. Hg.	Change, mm. Hg.
Barney	Normal	100	46	54	140	165	+25
Gunner	Normal	90	64	26	140	150	+10
Major	Normal	114	80	34	170	180	+10
Mike	Normal	72	52	20	130	125	− 5
General	Cardiac sympathectomy	120	76	44	140	160	+20
Ted	Cardiac sympathectomy	88	60	28	140	160	+20
Sargent	Cardiac sympathectomy	100	76	24	140	170	+30
Sally	Vagotomized	136	105	31	105	112	+ 7
Sambo	Vagotomized	148	132	16	110	120	+10
Katy	Vagotomized	120	110	10	115	135	+20
Colonel	Total cardiac denervation	80	80	0	120	135	+15

* *The slight restlessness of the animals under the conditions of the experiment raised the resting rate and prevented maximal inhibition.*

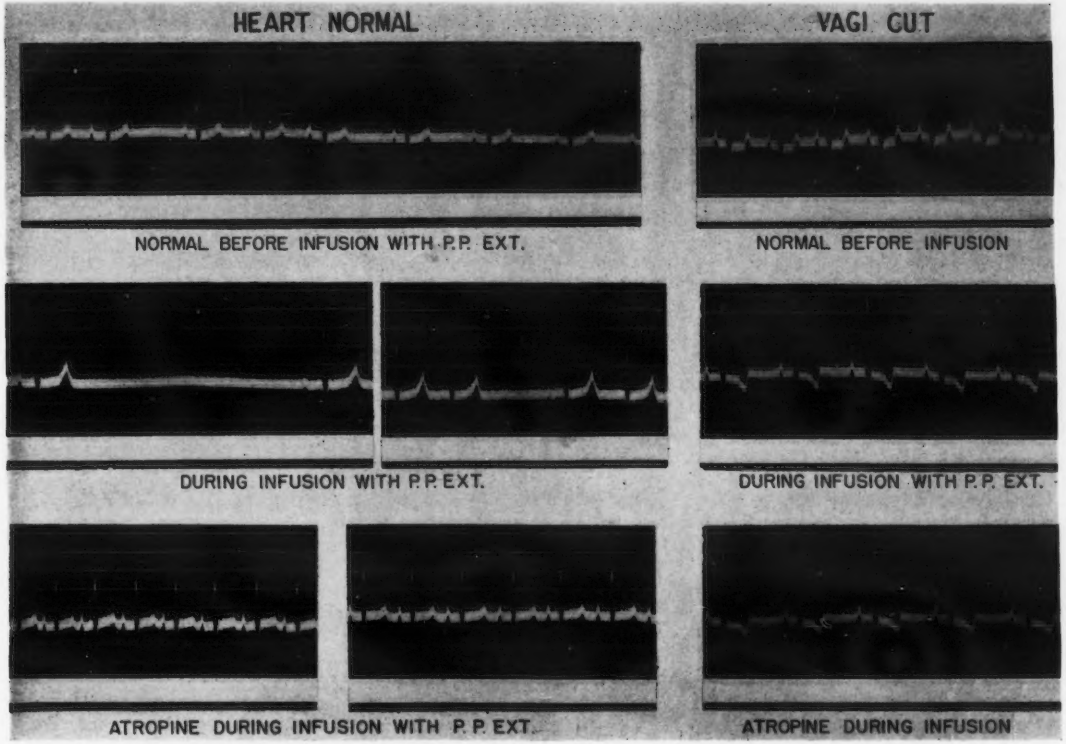

FIG. 5. *The effect of posterior pituitary infusion and the subsequent injection of atropine on the electrocardiogram of a dog before and after section of the vagi (Lead II).*

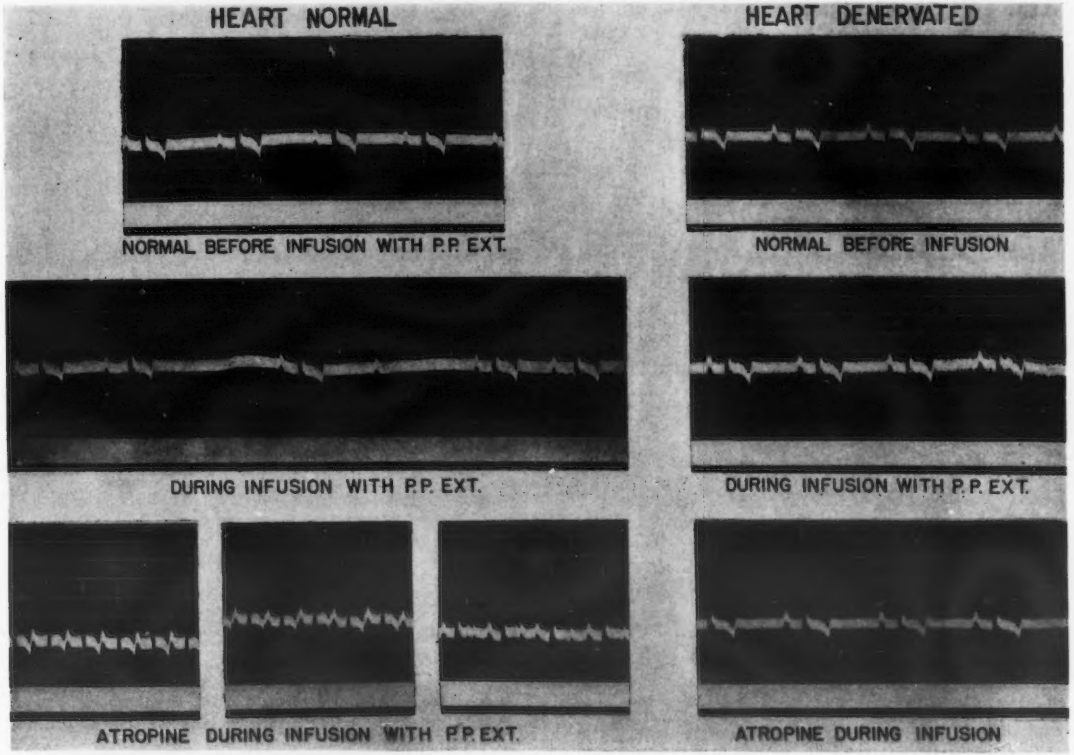

FIG. 6. *The effect of posterior pituitary infusion and the subsequent injection of atropine on the electrocardiogram (Lead II) of a dog before and after denervation of the heart. These records are from the animal that showed the maximal increase in basal heart rate after vagotomy.*

pressor effect. Anaesthetics, particularly chloretone, intensify the pressor action (3, 10). Coronary dilators such as ephedrine and adrenaline also increase the pressor effect of small doses and may nullify the depressor effect of large doses (8).

The effect of infusion of posterior pituitary extract on blood pressure was registered in seven normal unanaesthetized dogs. (For examples, see Table II, Fig. 4A). A pressor effect of 10 to 30 mm. of mercury was obtained within 30 sec. in six unanaesthetized dogs, and a fall of 5 mm. in one. The maximal cardio-inhibition followed the maximal pressure rise, but the heart was slowed even in the animal that showed the depressor effect. The pressure slowly fell with continued infusion, sometimes reaching the pre-infusion level. The bradycardia was maintained however, and the rate did not return to the resting level until 20 min. after cessation of infusion. These results indicate that the vagal effect does not depend on a depressor reflex.

B. Dogs with Thoracic Sympathectomy

Thoracic sympathectomy, with removal of stellate ganglia, was done in four dogs. This did not alter the resting-heart rate of 60 to 90 per minute. Two weeks after the final operation, posterior pituitary infusions were given. This produced an inhibition indistinguishable from that in the normal animal (Table I), with normal blood pressure elevation (Table II) and electrocardiographic effects, and with normal recovery time. Bacq and Dworkin (1) found that single injections of pitressin in the completely sympathectomized cat produced the same inhibition as in the normal cat, although the recovery time was prolonged.

Atropine injected during the bradycardia produced by infusion caused the typical tachycardia seen in the normal dog.

C. Vagotomized Dogs

Samaan (13) and others found that section of both cervical vagus nerves in the anaesthetized dog is followed by extreme cardiac acceleration. A similar increase in basal heart rate has been reported for the unanaesthetized cat (1, 9).

In this investigation bilateral vagotomy was done in three dogs whose response to posterior pituitary infusion, with and without atropine, had been established. Two of these had higher resting-heart rates than had most of the experimental animals. No change in heart rate occurred after section of one vagus. After double vagotomy one animal showed no change in basal heart rate (Fig. 1), the second showed a slight rise, and the third, a considerable acceleration (Table III). This third animal had respiratory irregularities probably due to inability to accommodate in the short interval (six days) between section of the two nerves.

TABLE III

THE EFFECT OF POSTERIOR PITUITARY EXTRACT ON HEART RATE BEFORE AND AFTER VAGOTOMY

Dog Sally			Dog Katy			Dog Sambo		
Resting heart rate per min. on different days	Minimal infusion rate per min.	Average fall in heart rate	Resting heart rate per min. on different days	Minimal infusion rate per min.	Average fall in heart rate	Resting heart rate per min. on different days	Minimal infusion rate per min.	Average fall in heart rate
Before vagotomy			Before vagotomy			Before vagotomy		
102	48		110	60		90	40	
100	56		94	43		74	36	
84	56		124	56		84	44	
102	48		96	60		66	34	
88	56	42			51	74	37	39
After unilateral vagotomy			After unilateral vagotomy					
102	83		102	54				
96	68		102	56				
90	68	23			47			
After double vagotomy			After double vagotomy			After double vagotomy		
104	92		132	108		138	122	
100	86		124	104	27	148	128	18
90	76							
96	80							
98	80							
134	118	15						

The Effect of Posterior Pituitary Infusion on the Heart Rate

After double vagotomy the inhibition produced by posterior pituitary infusion was considerably less than that which occurred in the normal animal (Table III). Its onset was delayed and did not reach a maximum until 20 min. after the start of infusion (Fig. 1). It was then maintained but gradually ceased after withdrawal. Blood pressure changes during infusion were similar to those obtained in the normal animal (Table II, Fig. 4B). Infusion produced the following electrocardiographic changes (Fig. 5): lengthening of the P–R interval, inversion of T wave, increase in T voltage. There was no grouping of beats. The inversion of the P wave reported by Melville (7) was not seen.

The Effect of Atropine on Posterior Pituitary Inhibition

Atropine has been reported to cause cardiac acceleration after double vagotomy in the cat (9) and the dog (12).

We were not able to demonstrate this at any time in three doubly vagotomized dogs, either immediately after section or two weeks later. When atropine was given while the heart was inhibited by pituitary infusion, there was no acceleration (Fig. 1). Given before infusion, atropine did not inter-

fere with the inhibitory action of posterior pituitary extract. Atropine did not alter the electrocardiogram of the vagotomized dog under influence of posterior pituitary extract (Fig. 5).

It is significant that in the dog with a sympathetic cardiac denervation only, posterior pituitary infusion still exercises an inhibition on the heart, even in the presence of atropine.

D. Dog after Unilateral Vagotomy and Bilateral Thoracic Sympathectomy

Several experiments were done on one animal after recovery from single vagotomy and double thoracic sympathectomy.

Infusion with posterior pituitary extract produced a slowing of the heart equivalent to that produced when the same heart had its normal innervation.

Atropine caused the same acceleration as was produced before the partial denervation, and overcame the bradycardia produced by posterior pituitary infusion just as in the normal dog.

E. Animals with Complete Denervation of the Heart

Bacq and Dworkin (1) produced no change in heart rate with single injections of pitressin in the cat immediately after total denervation, but observed a considerable acceleration of the chronically denervated heart.

TABLE IV

THE EFFECT OF POSTERIOR PITUITARY EXTRACT ON HEART RATE BEFORE AND AFTER COMPLETE DENERVATION OF THE HEART

Dog Barney			Dog Colonel		
Resting heart rate per min. on different days	Minimal infusion rate per min.	Average fall in heart rate	Resting heart rate per min. on different days	Minimal infusion rate per min.	Average fall in heart rate
Before denervation			Before denervation		
104	38		90	54	
90	36		90	42	
84	34		68	34	
102	40		62	31	
86	36	56			37
Both sympathetic chains removed, 1 vagus cut					
82	36				
88	48				
82	48	40			
Heart completely denervated			Heart completely denervated		
92	94		92	88	
96	102		82	78	
94	90	Nil			4

Experiments were done on two animals after operative recovery from total cardiac denervation. Infusion with posterior pituitary extract produced no characteristic slowing of the heart rate in five experiments in our dogs, 1 to 10 days after total denervation (Table IV, Fig. 2). It caused the characteristic rise in blood pressure (Fig. 4C). Denervation did not alter the electrocardiogram from the normal, and infusion now made no change in it (Fig. 6).

Atropine alone or in combination with pituitary infusion had no effect on the heart or electrocardiogram (Fig. 6).

Discussion and Conclusions

Our results indicate that the inhibitory action of physiological doses of pituitary extract in the unanaesthetized dog is *solely* through the cardiac nerves. This is contrary to the opinion of several investigators who conclude that posterior pituitary extract acts, not only on the vagus mechanism, but also by direct myocardial depression, or coronary arterial constriction, or both.

Our experiments indicate that both sympathetic and vagus fibres are involved, since after vagotomy the extract is still able to cause some inhibition, but only so long as the sympathetic innervation is retained. Cardioinhibitory fibres have been demonstrated in the sympathetic supply to the dog heart (5); these are not affected by atropine. In the normal animal posterior pituitary inhibition is probably mainly through the vagus, since interruption of the sympathetic supply does not diminish it.

It is unlikely that the nervous inhibition is due to a carotid sinus-depressor reflex, since it may be initiated with no rise or only a slight rise in blood pressure (Table I).

Our experiments do not demonstrate whether the stimulation of inhibitory fibres is central or reflex from the heart. If reflex, afferent as well as efferent fibres must exist in both sympathetic and parasympathetic pathways.

We do not deny that posterior pituitary extract can cause coronory arterial constriction, by direct action, but this does not seem to be a primary factor in causing inhibition, for total denervation prevents the inhibitory effect of the extract. If constriction were to activate sensory receptors in the walls of the arteries, this might produce reflex inhibition which would be abolished by denervation.

Since total denervation abolishes the inhibitory effects, it is unlikely that posterior pituitary extract acts by myocardial depression. Support to this view is provided by the observation that the typical electrocardiographic signs that accompany the inhibition of the normal heart are abolished when the extract is infused after total cardiac denervation.

Our results with the dog are in partial agreement with those of Bacq and Dworkin with the cat, in which complete denervation abolished the inhibitory effect of pitressin. They found, however, that pitressin produced acceleration of the chronically denervated heart. This was never observed in our dogs either immediately after total denervation, or for two weeks thereafter.

References

1. BACQ, Z. M. and DWORKIN, S. Am. J. Physiol. 95 : 605-613. 1930.
2. CANNON, W. B., NEWTON, H. F., BRIGHT, E. M., MENKIN, V., and MOORE, R. M. Am. J. Physiol. 89 : 84-107. 1929.
3. GRUBER, C. M. J. Pharmacol. 36 : 155-172. 1929.
4. GRUBER, C. M. and KOUNTZ, W. B. J. Pharmacol. 39 : 275-299. 1930.
5. HERMANN, H., JOURDAN, F., and FROMENT, R. Compt. rend. soc. biol. 128 : 673-676. 1938.
6. MELVILLE, K. I. J. Pharmacol. 47 : 355-363. 1933.
7. MELVILLE, K. I. J. Pharmacol. 64 : 86-110. 1938.
8. MELVILLE, K. I. and STEHLE, R. L. J. Pharmacol. 42 : 455-470. 1931.
9. MOORE, R. M. and CANNON, W. B. Am. J. Physiol. 94 : 201-208. 1930.
10. RAGINSKY, B. B., ROSS, J. B., and STEHLE, R. L. J. Pharmacol. 38 : 473-480. 1930.
11. RESNIK, W. H. and GEILING, E. M. K. J. Clin. Investigation, 1 : 217-238, 239-245. 1925.
12. ROGERS, F. T. Am. J. Physiol. 53 : 15-24. 1920.
13. SAMAAN, A. J. Physiol. 83 : 332-340. 1935.
14. WAKIM, K. G., HERRICK, J. F., BALDES, E. J., and MANN, F. C. J. Lab. Clin. Med. 27: 1013-1022. 1942.

THE PRODUCTION OF CANNED PRECOOKED CHICKEN[1]

By E. J. Reedman[2]

Abstract

Three classes of raw dressed chicken were processed by open and pressure precooking methods, and tested by a consumer taste panel. Canned chicken of good quality was obtained from even the lowest grade of raw dressed chicken used, but the higher grades of raw material produced packs of superior quality. However, such quality was dependent on efficient processing. The results suggest that the quality of Canadian canned chicken could be improved by the uniform adoption of pressure precooking methods by the industry. The use of pressure precooking would also assist in the production of a more uniform pack, because of lower moisture content of the cooked meat, smaller volume of broth and shorter cooking periods. Control measures are necessary to ensure uniformity of product, particularly in such attributes as the weight of meat packed, the concentration of broth used, and the strength of jelly in the final product. Suitable methods and apparatus are described.

Introduction

As a result of the increased war-time demand for eggs, Canadian production of canned precooked chicken (also known as canned sliced or canned boned chicken) has developed considerably in recent years. A survey of commercial products indicated that quality improvement was possible in many, while a lack of uniformity even within the same brand definitely pointed to a need for closer control in manufacture. In the present investigation, therefore, attention was directed towards improvement of the quality of Canadian canned chicken, and the production of a more uniform pack.

Factors Affecting Quality of Product

Grades of raw material and the cooking methods used were considered to be the most probable factors having appreciable effects upon the quality of canned precooked chicken.

Materials

The canning industry naturally prefers to use the least expensive (i.e., lowest grade) raw material available; provided that it produces a finished product of desirable quality. Since the top grades for chicken set by the Canadian Standards (1) are strongly influenced by such factors as the colour of the fat and the symmetry of the bones, this attitude of the canners appeared to merit consideration, particularly as both bones and fat are removed before the chicken is canned.

In the present investigation, three classes of raw dressed chicken were used: Class *A* was a composite of the three top grades, namely, Special

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Milk Fed, *A* Milk Fed, and *B* Milk Fed; Classes *B* and *C* were equivalent to standard grades *B* and *C;* however, raw material that had been graded down because of appearance factors was regraded for canning purposes.

Methods

The relative merits of open and pressure precooking also appeared to warrant investigation. Open precooking is the most widely used commercial method. Since the maximum temperature that can be reached is boiling point, prolonged cooking periods are required (60 to 90 min.). It is necessary to cover the meat in the kettle with water, which produces a large volume of broth, although this can be reduced by cooking several successive lots of meat in the same broth, or by concentrating. However, in pressure precooking, only sufficient water to generate steam need be added; this amounts to about 1 qt. per 25 lb. of meat. Also there is no loss from the kettle and a minimum exposure to oxidation. Since temperatures of 121° C. (250° F.) or even higher may be used, rapid cooking is obtained (15 to 30 min.).

Procedure and Results

Each of the three classes of raw material was precooked by both the open and pressure methods described, packed at the same weight of meat and strength of jelly in 7-oz. cans, and canned by the same process. This series of six packs was produced in a commercial establishment. A taste panel consisting of 30 members was used to assess the quality of the finished product. In this test comparisons between all packs were made by tasting two samples at a time.

Table I shows that, when the results are averaged over the entire experiment, neither the class of raw material nor the method of precooking had significant effects on eating quality. However, when the classes are considered in detail, as averaged over both precooking methods, it is evident that Class *C* chicken was significantly inferior to Classes *A* and *B*, and that no difference could be distinguished between *A* and *B* classes. Detailed analysis of the differential quantities shows, however, that when pressure precooking was used, Class *A* chicken was distinguished as being significantly superior to the other classes.

These results indicate that three classes of chicken can be distinguished when prepared by the pressure precooking methods used here, but only two classes can be distinguished when open precooking is used. The higher quality of first grade raw material can be retained only by the use of pressure precooking. It has also been shown that pressure precooking results in a higher retention of vitamin C (3).

Factors Affecting Uniformity of Product

Since uniformity is essential in maintaining brand consistency, it was considered desirable to apply control measures wherever feasible. Factors that appeared amenable to control were: the proportion of meat to jelly, the concentration of broth used, and the strength of jelly in the final pack.

TABLE I

RESULTS OF QUALITY TESTS ON CANNED CHICKEN

Ratings assigned by a flavour test panel of 30 members

Cooking method	Class of chicken			Total	$A + B$
	A	*B*	*C*		
Open	−10	8	−17	−19	− 2
Pressure	36	5	−22	19	41
Total	26	13	−39	0	39

Analysis of variance

Source of variance	Degrees of freedom	Mean square
Treatments	5	5.02
Class	2	6.57
C vs. $A + B$	1	12.68*
A vs. B	1	0.47
Cook	1	2.67
Class × cook	2	4.89
C vs. $(A + B)$ × cook	1	2.60
A vs. B × cook	1	6.67
A vs. $(B + C)$ × cook	1	9.26*
B vs. C × cook	1	0.01
Error (b)†	145	2.33*
Error (a)	300	1.75

† *Error (b) used to test the significance between treatments and between its various components.*
* *Exceeds 5% level of statistical significance.*

Ratio of Meat to Jelly

The quality of a canned food is affected by the quantity of solids or concentrate in proportion to water or other filling material present. For canned chicken, 50% by weight of meat appears to be a reasonable lower limit. The use of pressure precooking enables all the broth produced to be packed. Pressure and open precooked meat contain 60 to 65% and 70 to 75% moisture, respectively, and since the retorting process has been found to reduce the higher moisture content to approximately 60 to 65%, adjustments should be made when packing open precooked meat.

A separation device suitable for checking weights of meat and jelly should be capable of being heated under pressure to liquefy the jelly, and vapour-tight to prevent the loss of volatile materials. Such an apparatus is shown in Fig. 1. The apparatus consists essentially of upper and lower receptacles joined with an air-tight gasket, and upper and lower plates that can be used to draw the receptacles together by means of bolts and wing nuts. The bottom of the upper receptacle consists of 60-mesh stainless steel screening. The previously weighed receptacles are assembled, the contents of a can

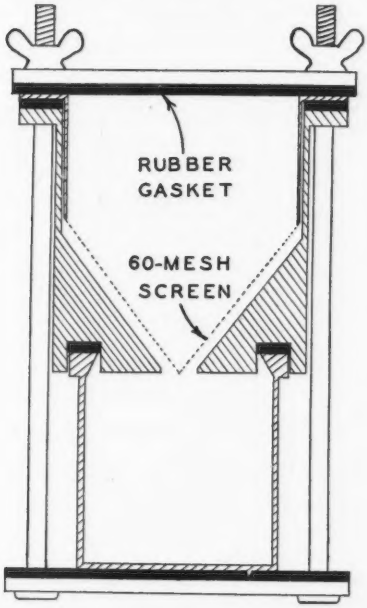

FIG. 1. *Apparatus used for separating meat and jelly in canned chicken.*

weighed into the upper receptacle, and the entire apparatus heated under pressure to liquefy the jelly. After heating, the separation apparatus is cooled, the receptacles are weighed, and the weights of meat and jelly determined.

It was found that a 30-min. heating period at 121° C. (250° F.) with a subsequent cooling of 30 min. in cold water, gave a complete separation of a 7-oz. net weight pack. Moreover, this procedure gave a meat of approximately the same moisture content as that after retorting. Table II gives typical data on the proportions of meat and jelly obtained in laboratory and commercial tests.

Quality and Strength of Broth

In canned chicken the broth obtained from the meat during precooking is an important portion of the final product: not only does the broth contribute to the food value of the product, but it also contributes to flavour. The quality of broth depends upon the grade of raw material and the method of preparation. From the quality tests recorded in Table I, it appears that pressure precooked meat yields a broth of better flavour than that prepared by open precooking. Heating treatments used for concentrating or for dissolving gelling agents are deleterious to broth quality.

TABLE II

SEPARATIONS OF MEAT AND JELLY IN FINISHED CANS OF PRECOOKED CHICKEN

Class of sample	Net weight, oz.	Weight of meat, oz.	Weight of jelly, oz.	Moisture in meat after separation, %
Laboratory samples	7.0	3.5	3.5	63
	6.9	3.5	3.4	63
	7.0	3.6	3.4	64
	7.0	4.0	3.0	62
	7.0	4.1	2.9	63
Commercial samples	7.2	3.2	4.0	63
	7.3	3.5	3.8	64
	7.2	3.0	4.2	61
	7.1	2.9	4.2	62
	7.0	2.8	4.2	63
	6.9	1.9	5.0	62

It was considered desirable to measure broth concentration in terms of solids present. Two methods for measuring the total solids present were considered feasible, namely: the evaporation of a measured volume of broth to dryness and determination of the amount of solids remaining, or preferably, the measurement of the specific gravity of the broth and its correlation with actual solids found by the first procedure.

It has been commercial practice to separate the fat from the broth after precooking, since if fat is packed with the broth it will rise to the top in the can and give an undesirable flavour and appearance to the finished product. Measurements of broth concentration are therefore made after separation of the fat and before addition of salt, gelling agent, or other substance to the broth. Sodium chloride was determined by the Mohr method after ashing the samples in a muffle furnace. Solids corresponding to specific gravities from 0.990 to 1.040 at 50° C. (122° F.) were measured, enabling determination of the correlation of specific gravity and solids in any broth likely to be encountered in commercial practice.

Table III shows specific gravities obtained in samples of broth prepared by both open and pressure precooking methods. From a large number of laboratory and factory measurements it was considered that the specific gravity should not fall below 1.000 or 1.010 at 50° C. (122° F.) for open and pressure precooked broths, respectively, if a desirable strength of broth was to be packed.

Quality and Strength of Jelly

Meat broth contains natural gelatine which contributes to the strength of the final jelly, but such natural gelatine has a tendency to hydrolyse or break down in the commercial sterilization of the product after packing. Consequently it is now general practice to add further amounts of a gelling agent such as agar or gelatine to obtain a desirable strength of jelly. Agar should

TABLE III

SPECIFIC GRAVITY AND SOLIDS CONTENT OF BROTHS FROM SAMPLES OF PRECOOKED CHICKEN[1]

Method of precooking	Specific gravity at 50° C. (122°F.)	Weight of solids, oz. per gal.	Method of precooking	Specific gravity at 50° C. (122°F.)	Weight of solids, oz. per gal.
Pressure	1.021	15.9	Open	1.002	5.5
	1.0185	14.5		1.002	5.5
	1.0182	14.3		1.001	4.8
	1.0175	13.1		1.000	4.0
	1.0125	11.2		1.000	4.0
	1.011	10.0		1.000	4.0

[1] *These varying strengths of broth were obtained by different times of precooking. In commercial practice it is possible to obtain fairly uniform solids between batches. Open precooked broths had only one lot of meat cooked in them.*

not be added in a concentration of above 3% by weight in the final broth; two to two and one-half per cent is usually adequate (2). If gelatine is used the grade must be carefully chosen, to prevent excessive hydrolysis on retorting. Typical data on the solids content of the jelly are given in Table IV. It is of importance to note that during retorting additional solids come out of the meat into the broth.

TABLE IV

SOLIDS IN THE BROTH AND JELLY OF CANNED CHICKEN

Broth after precooking		Broth after addition of agar and salt		Final jelly after retorting	
Specific gravity at 50° C. (122°F.)	Solids content, oz. per gal.	Specific gravity at 50° C. (122°F.)	Solids content, oz. per gal.	Specific gravity at 50° C. (122°F.)	Solids content, oz. per gal.
1.009	8.6	1.013	12.1	1.025	17.5
1.010	9.5	1.020	15.6	1.033	21.0
1.010	9.5	1.020	15.6	1.030	20.0
1.011	10.0	1.0205	15.8	1.033	21.0
1.011	10.0	1.021	15.9	1.030	20.0

The final jelly strength may be tested as follows: the jelly and meat are separated in the apparatus described and the jelly allowed to solidify in the lower receptacle. (Separation weakens the jelly somewhat, but since this is a constant factor it can be disregarded.) The jelly is conditioned for three hours at 7° C. (45° F.). The apparatus used for determining jelly strength (Fig. 2) consists essentially of an oil-damped trip scale with the pointer extended to a length of one foot. The receptacle containing the jelly is placed on one pan and the scale balanced at zero by adjustment of the beam weights. A cone-shaped plunger mounted above the jelly is so adjusted that its tip just touches the surface of the jelly. Mercury is run into a container

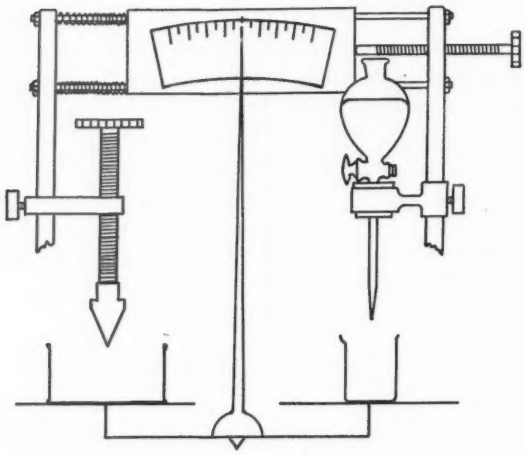

FIG. 2. *Device for testing jelly strength.*

on the other pan to force the jelly against the plunger a standard distance, as shown by the deflection of the pointer. Thus jelly strength is measured by the resistance of the jelly to the penetration of the plunger, and is expressed as grams of mercury. Table V shows typical results obtained with this device.

TABLE V

MEASUREMENTS OF JELLY STRENGTH

Class of sample	Description	Observations on jelly	Jelly strength, gm. mercury	
			Range	Average
Laboratory samples	1% agar in broth	Did not gel	—	—
	2% agar in broth	Fairly firm jelly	13.0—15.7	15.1
	2% agar in broth	Fairly firm jelly	15.7	15.7
	2.5% agar in broth	Firm jelly	15.6—35.5	26.6
Commercial samples	—	Very weak jelly	5.3—10.1	8.0
	—	Very weak jelly	6.5— 6.9	6.6
	—	Very weak jelly	6.2— 7.2	6.7
	—	Weak jelly	9.7—12.5	10.7
	—	Fairly firm jelly	14.4—14.9	14.5
	—	Firm jelly	25.5—32.3	28.2

Acknowledgments

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Laboratories, who aided in the preparation of the manuscript, and to Mr. Leonard Buckby, Laboratory Assistant, who prepared the figures.

References

1. CAN. DEPT. AGR. Regulations under the provisions of the Live Stock and Live Stock Products Act, Chapter 47, of the Statutes of Canada, 1939, respecting the Grading and Marking of Dressed Poultry. (As published in the Canada Gazette, April 3, 1943.)
2. REEDMAN, E. J. Unpublished data.
3. REEDMAN, E. J., BUCKBY, L., and LEGAULT, R. T. Can. J. Research, D, 20 : 230-234. 1942.

DRIED WHOLE EGG POWDER

VII. EFFECT OF TEMPERATURE AND MOISTURE ON THE BACTERIAL CONTENT OF LIQUID AND DRIED EGG[1]

By N. E. Gibbons[2] and C. O. Fulton[2]

Abstract

The bacterial content of liquid egg increased rapidly after about six hours at 20° C., 12 hr. at 15.6° C., 25 hr. at 11.1° C., and two to three days at 7.2° C. At 3.3° C. there was little change for five to six days, followed by a very gradual increase.

The bacterial content of dried egg powder was influenced by the number of bacteria in the melange, the drying temperature, the rate of cooling, the storage temperature, and the moisture content. Low drying temperature and rapid cooling of the powder favoured survival. On storage the bacterial mortality increased with increasing time and temperature. At temperatures above 30° C. the death rate seemed to be proportional to changes in temperature. At 7.2° C. and lower the majority of organisms survived eight months' storage. Up to 8.6%, moisture content had little effect on bacterial survival. At moisture levels above 5% there was an increase in the number of moulds, particularly at 23.9° and 32.2° C.

Introduction

Although there is as yet no direct evidence that any correlation exists between bacterial content and the quality of dried egg powder, the number of bacteria present in the powder gives some indication of the sanitary conditions prevailing in the breaking and drying plants. Information on the effect of plant practices on survival or increase of organisms during the storage and drying of melange and the cooling and storage of powder, presented here, was collected during actual plant operation and in the laboratory. Most of the data is concerned with dried egg. However, some experiments on the effect of temperature on the bacterial content of the egg liquid are also included, since the load in the liquid determines to a large extent the bacterial content of the powder.

Methods

The number of organisms in liquid egg was estimated by shaking 10 ml. of the melange with 90 ml. of water and making the appropriate dilutions.

The method of making counts on powder has been described (3). Plates were poured using proteose-peptone, tryptone agar, and counts were made after incubating for three days at 37° C.

Comparisons were made between the above agar and the tryptone glucose agar without the addition of milk that is usually recommended for the examination of egg products (1). Duplicate samples from 20 carlots were plated on

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both media and incubated for three days at 32° C. and 37° C. An analysis of variance indicated no significant difference between the counts obtained on the two media. However, there was a significant difference in the number of organisms at the two incubation temperatures. This was due to the fact that on about half the samples the count at 32° C. was significantly higher than at 37° C. It would appear therefore that the flora of egg powders may differ and that the lower incubation temperature favours the development of certain of these organisms.

Results

Liquid Egg

In an initial experiment to determine the temperature at which liquid egg may be held with safety, melange prepared from commercial eggs was stored for periods up to 21 days at 3.3°, 7.2°, 20°, 23.9°, and 37° C. (38°, 45°, 68°, 75°, and 98° F.).

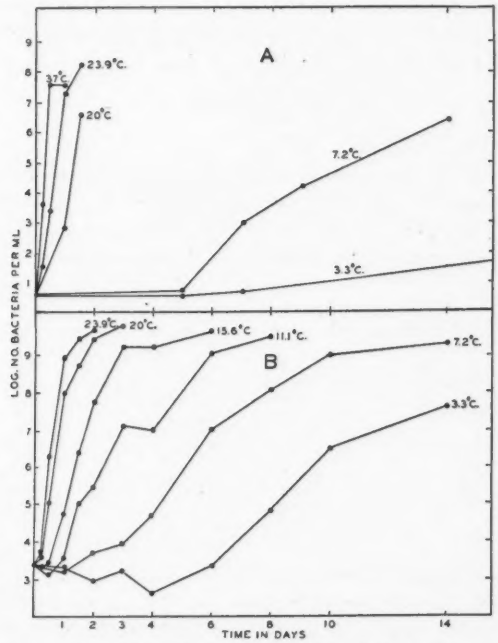

Fig. 1. *Increase in number of bacteria in liquid egg held at various temperatures.*

Although the original count was less than 10 organisms per ml., the count rose rapidly at room temperature and above (Fig. 1A). However, at 7.2° C. there was no appreciable increase for five days and the pH changed little from that of the original (7.5). The melange stored at 37° C. coagulated in 24 hr. (final pH 5.4), in 36 hr. at 23.9° C. (pH 5.7) and in 48 hr. at 20° C. (pH 5.7).

Since it was considered possible that growth might be rapid at temperatures slightly above 7.2° C., a second experiment was carried out using melange with a somewhat higher initial count. This material was incubated at 3.3°, 7.2°, 11.1°, 15.6°, 20°, and 23.9° C. for periods up to 14 days. It was again observed that the rate of bacterial development increased as the temperature increased (Fig. 1B). At 7.2° C. a fairly rapid increase took place after a lag of about three days and after approximately six days at 3.3° C.

It would appear that a different flora was present in this lot of melange since even at 23.9° C. with a bacterial content of some thousand million per ml. at 24 hr., the pH dropped to only 6.7 and then increased to 7.1. This drop and rise in pH was noted at all temperatures above 11.1° C. The more rapid growth at lower temperatures than in the previous experiment would also indicate a different flora.

From the above results it would appear that under normal sanitary and storage conditions egg melange can be held for 24 hr. and possibly 48 hr. at 7.2° C. without the bacteria increasing appreciably. At a temperature of 11.1° C. melange cannot be held safely for even 24 hr. and at 20° C. for 12 hr. unless the initial count is extremely low.

Dried Egg Powder

Effect of Temperature of Drying

The temperature at which the liquid egg is dried is an important factor in determining the quality of the powder produced (7). Within limits, the lower the drying temperature the better the quality of the powder, but, as might be expected, more bacteria survive. This is shown in Table I, where

TABLE I

Effect of drying temperature on the bacterial content of egg powder

Plant	Outlet temperature, ° C.	No. of organisms per gram	Outlet temperature, ° C.	No. of organisms per gram
A	65	8900	51.7	26,500
B	68.3	26,000	54.4	130,000
C	57.2	112,000	52.2	338,000

the bacterial content of powder dried at two different temperatures in three plants is recorded. A decrease of from 4° to 15° C. in the temperature of the outlet air is accompanied by a three- to fivefold increase in the number of bacteria in the resulting powder.

Effect of Cooling

Rapid cooling of the powder after leaving the drier is important if high quality is to be maintained (4). Slow cooling, on the other hand, will usually result in powders with lower bacterial counts. For instance, it has been observed that samples removed from the top and bottom layers of powder,

collected in the bottom of a box type drier having an outlet temperature of 51.8° C., had different counts. The bottom layer, which had been exposed to this temperature for about an hour, had a count of 6700 per gram as compared with 32,000 in the top layer which had been exposed for a shorter time.

In an initial experiment to study the effect of cooling rates, changes in the bacterial content of the powder in two barrels held at 5.6° C. were followed. The barrels were filled with powder at 40.6° C., headed immediately, and placed at 5.6° C. for four days. The powder near the outside of the barrel was below 21° C. in six hours, 9.4° C. in 24 hr., and 5.8° C. in 48 hr. In the centre the temperature remained at 40.6° C. for six hours and dropped to 36° C. after 24 hr. After two and a half days the centre was just below 21° C. and after four days, 12.2° C. The barrels were stored a further seven days at 26.7° C. before sampling. The bacterial content of one barrel decreased from an initial count of 17,000,000 per gm. to 62,000 per gm. in the centre and 190,000 at the outside. In the second barrel an initial count

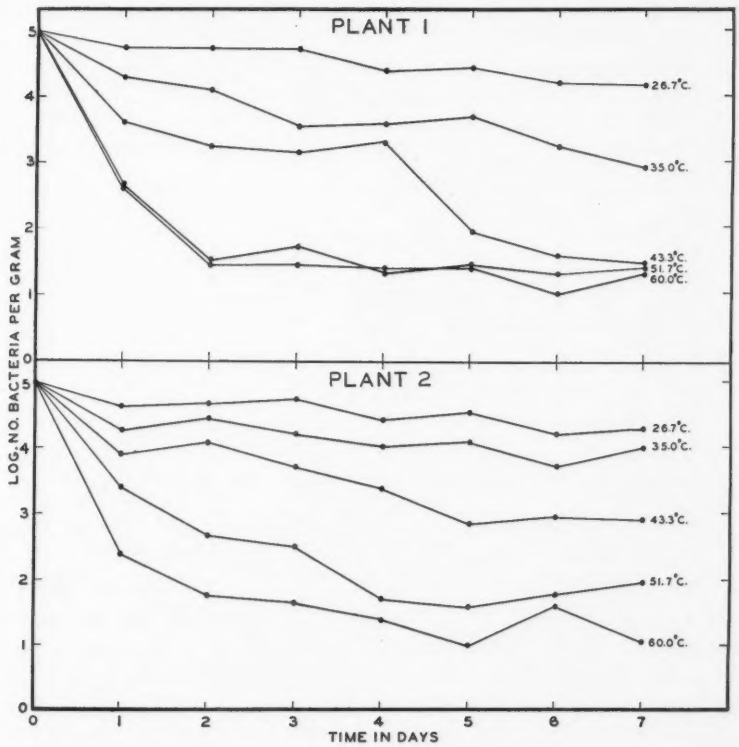

FIG. 2. *Changes in the bacterial content of egg powder held at 26.7°, 35.0°, 43.3°, 51.7°, and 60° C. for seven days.*

of 10,000,000 per gm. decreased to 30,000 in the centre and 190,000 at the outside. This differential effect indicated that cooling is an important factor in determining the bacterial content of dried egg powder.

Since cooling rates are difficult to control in practice, pertinent information was obtained by holding powders from two plants at 26.7°, 35°, 43.3°, 51.7°, and 60° C. for 3, 6, 12 hr. and one, two, three, four, five, six, and seven days (4).

The effect on the bacterial content is shown in Fig. 2. At 60° C. over 99% of the organisms were destroyed during the first day. At 51.8° C. the greatest reduction also took place during the first day. However, in the same time the quality of the powder deteriorated considerably (4). At 43.3° and 35° C. there was a gradual but definite decrease with time but at 26.7° C. there was only a very slight decrease over the seven days. It is therefore evident that if the powder were cooled rapidly enough to preserve quality the majority of the organisms surviving the drying process would also be preserved.

Effect of Storage Temperature

Powder from one plant was stored for 36 wk. at 0.56°, 7.2°, 20°, and 37° C. and at 55° C. for 10 wk. The results are presented in Fig. 3. At 0° C. there was little change in the bacterial content. At temperatures of 7.2° C. and higher, the rate of bacterial decrease was proportional to the increase in

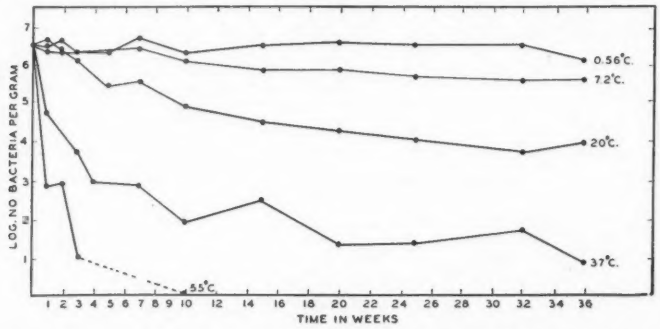

FIG. 3. *Changes in the bacterial content of egg powder held at 0.56°, 7.2°, 20°, 37°, and 55° C. for periods up to 36 wk.*

temperature. At 37° C. there was a decided decrease during the first four weeks, with a more gradual change thereafter. At 55° C. the powder was practically sterile after four weeks since less than five organisms per gm. were present. At the 10th week it was found, by the dilution method, that there was about one organism per gm. These were spores.

In a second storage experiment powders from three producers were stored at 7.2°, 15.6°, and 23.9° C. for eight months and at 32.2° C. for three and a half months. The results (Fig. 4) in general are in agreement with those of

the preceding experiment. The greater reduction in bacterial content at 32.2°, as compared with 23.9° C. and lower, is of interest since a similar observation was noted for changes in quality (6).

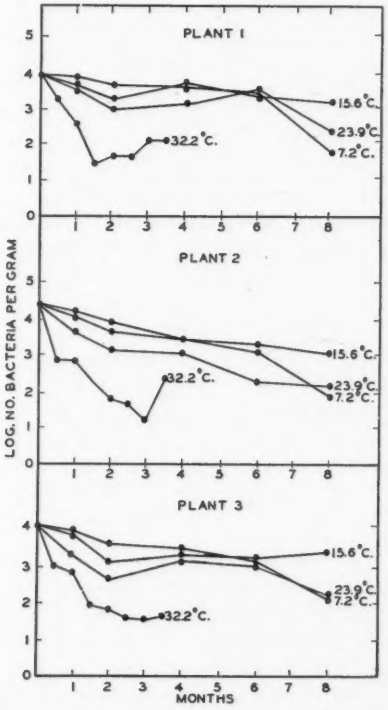

FIG. 4. *Effect of storage at 7.2°, 15.6°, 23.9°, and 32.2° C. for periods up to eight months on the bacterial content of egg powder from three plants.*

The moisture content of the powder stored in convolutely wound, paper-bodied containers held at 7.2° C. began to increase about the fourth month (6). By the sixth month the moisture level had increased about 2% and by the eighth month about 9%. The decrease in the bacterial content of this powder was apparently linked with the change in moisture content.

Effect of Moisture Content

Powder from a commercial drier was adjusted to moisture levels of 3.07, 3.7, 5.3, and 8.6% (5) and stored at 7.2°, 15.6°, 23.9°, and 32.2° C. At 32.2° C. examinations were made semimonthly for two months and at the lower temperatures after one, two, four, and six months.

The results are shown in Fig. 5. Although an analysis of variance of the data indicated that there was a significant difference in the bacterial content of the powders with different moisture contents it is evident that much of this is due to differences in the original bacterial population of the powders. The

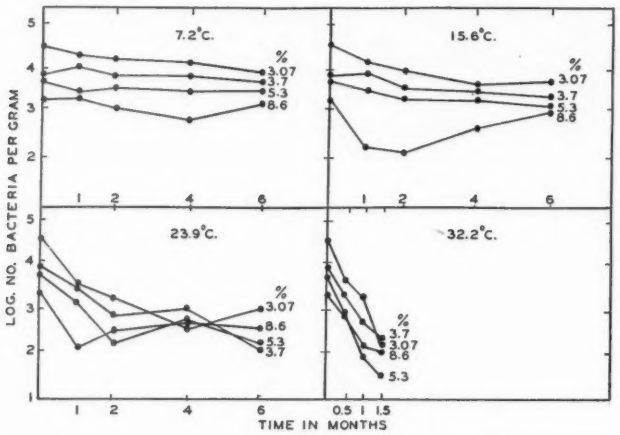

FIG. 5. *Effect of 3.07, 3.7, 5.3, and 8.6% moisture content on the bacterial content of egg powder stored at four temperatures.*

powder adjusted to 3.07% moisture was handled considerably while bringing it to a lower moisture level which may have been responsible for the high count. It is not known why raising the moisture content to 8% resulted in a lowering of the bacterial content although, as mentioned in the preceding section, the bacterial content of powders held at 7.2° C. began to decrease when the moisture content began to increase. Changing moisture levels appears to have more effect on the bacterial content than constant levels. It would seem that at the levels studied, once the moisture content has been adjusted, temperature is the more important factor in determining the change in bacterial content.

In contrast to the behaviour of bacteria there was an increase in the number of moulds at 5% and particularly at 8% moisture. This was more noticeable at 23.9° and 32.2° C. but in some instances numerous moulds were observed on the plates of powders stored at 7.2° C.

The same behaviour was noted in a study of containers. In one container in which the moisture content of the powder increased from 3.6 to 7.5% the number of moulds increased and the number of bacteria decreased. In another instance where the moisture content increased from 3.6 to 16% in one month the powder was a mass of mould, and bacteria could not be detected on the plates. The storage temperature was 23.9° C. in both cases.

These data are not altogether in agreement with published observations that in 60 days at 30° C. the decrease in numbers of bacteria was least at about 5% moisture content and greater at the lower levels and at higher levels up to 10% (2). In the present studies at 32.2° C. it has been found that there is a steady decrease in numbers at all constant moisture levels used. The irregular results obtained at the higher temperatures with the powder containing 8.6% moisture are probably fortuitous.

It has been noted, however, that when the moisture content changed during storage, as in the work of Stuart *et al.*, decreases in the numbers of bacteria were found. Such differences, however, occur in the types of organisms present in different lots of powder that it is difficult to generalize from a single experiment. Stuart *et al.* (2) did not find an increase in mould count at moisture levels below 10%. In the present study an increase in the number of moulds, usually accompanied by a decrease in the number of bacteria, was noted at all moisture levels at 32° C., although separate mould counts were not made.

Acknowledgment

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References

1. American Public Health Association. Standard methods for the examination of dairy products. 8th ed. New York. 1941.
2. Stuart, L. S., Hall, H. H., and Dicks, E. E. U.S. Egg Poultry Mag. 48 : 629-633, 658. 1942.
3. Thistle, M. W., Pearce, J. A., and Gibbons, N. E. Can. J. Research, D, 21 : 1-7. 1943.
4. White, W. H. and Thistle, M. W. Can. J. Research, D, 21 : 194-202. 1943.
5. White, W. H. and Thistle, M. W. Can. J. Research, D, 21 : 211-222. 1943.
6. White, W. H., Thistle, M. W., and Reid, M. Can. J. Research, D, 21. In press. 1943.
7. Woodcock, A. H. and Reid, M. In preparation.

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